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Department of Fisheries and the Environment
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Relative Resistances of Three Transferrin Genotypes of Coho Salmon (*Oncorhynchus kisutch*) and Their Hematological Responses to Bacterial Kidney Disease

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SUZUMOTO, B. K., C. B. SCHRECK, AND J. D. MCINTYRE. 1977. Relative resistances of three transferrin genotypes of coho salmon (*Oncorhynchus kisutch*) and their hematological responses to bacterial kidney disease. J. Fish. Res. Board Can. 34: 1–8.

Juvenile coho salmon (*Oncorhynchus kisutch*) of three transferrin genotypes (*AA*, *AC*, and *CC*) were experimentally infected with the causative agent of bacterial kidney disease (BKD) and mortalities observed. Six experimental and control groups were used: 1) bacteria-infected + no Fe^{+3}; 2) bacteria-infected + low Fe^{+3} levels; 3) bacteria-infected + high Fe^{+3} levels; 4) saline control; 5) high Fe^{+3} control; and 6) low Fe^{+3} control. In all experimental groups, the *AA* genotype was the most susceptible to BKD and the *CC* genotype the most resistant. Addition of exogenous iron did not appear to increase the pathogenicity of the disease. Distribution and uptake of radioiron (^{59}Fe) in control and BKD-infected fish was studied. BKD-infected fish were found to have consistently higher levels of ^{59}Fe in whole blood, plasma, and liver than control fish. In control and experimental fish, spleen, head kidney, and liver tissues were found to accumulate ^{59}Fe, while white muscle and gall bladder tissues did not. The effect of BKD on certain hematological characteristics also was determined. Hemoglobin, packed cell volume, total plasma protein, plasma iron, and plasma corticoid levels all may have decreased with the progression of BKD.

SUZUMOTO, B. K., C. B. SCHRECK, AND J. D. MCINTYRE. 1977. Relative resistances of three transferrin genotypes of coho salmon (*Oncorhynchus kisutch*) and their hematological responses to bacterial kidney disease. J. Fish. Res. Board Can. 34: 1–8.

Des saumoneaux coho (*Oncorhynchus kisutch*) de trois génotypes de transferrine (*AA*, *AC* et *CC*) ont été infectés expérimentalement par l'agent pathogène de la maladie bactérienne du rein (BKD), et les mortalités ont été observées. Nous avons utilisé six groupes expérimentaux et groupes témoins : 1) infectés par la bactérie + sans Fe^{+3}; 2) infectés par la bactérie + faibles

[1]Cooperators are Oregon Department of Fish and Wildlife, Oregon State University, and U.S. Fish and Wildlife Service.

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niveaux de Fe^{+3}; 3) infectés par la bactérie + hauts niveaux de Fe^{+3}; 4) saline témoin; 5) témoin avec niveau élevé de Fe^{+3}; et 6) témoin avec bas niveau de Fe^{+3}. Chez tous les groupes expérimentaux, le génotype *AA* est le plus susceptible à la BKD, et le génotype *CC* le plus résistant. L'addition de fer exogène ne semble pas augmenter la sévérité de la maladie.

Nous avons étudié la répartition et l'assimilation de fer radioactif (^{59}Fe) chez les poissons témoins et chez les poissons infectés par la BKD. Les poissons infectés par BKD ont des niveaux uniformément plus élevés de ^{59}Fe dans le sang entier, le plasma et le foie que les poissons témoins. On constate, à la fois chez les témoins et chez les poissons expérimentaux, que les tissus de la rate, du rein antérieur et du foie accumulent ^{59}Fe, alors que les tissus du muscle blanc et de la vésicule biliaire ne l'accumulent pas.

Nous avons de plus déterminé l'effet de la BKD sur certaines caractéristiques hématolcgiques. L'hémoglobine, le volume de cellules concentrées, les protéines plasmiques totales, le fer plasmique et la corticoïde plasmique peuvent avoir tous diminué à mesure que la BKD progresse.

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EPIZOOTICS of corynebacterial kidney disease (BKD), a chronic infection of salmonid fishes, frequently has resulted in high fish mortalities in hatcheries (Sniezko and Griffin 1955). Studies in our laboratory (unpublished data) demonstrated that agglutinating antibody titers for BKD were higher in certain transferrin genotypes of coho salmon (*Oncorhynchus kisutch*) returning to Big Creek Hatchery, Oregon, than in others. These results indicate that there may be a differential genotypic resistance to the disease. Transferrin, an iron binding plasma protein, has been implicated in resistance to many infectious diseases (Sussman 1974) and, consequently, could have a genetic basis for resistance to BKD.

In this report, we describe the results of a study designed to determine whether or not three transferrin genotypes of coho salmon differed in their resistance to BKD. Growth and virulence of many pathogenic bacteria in mammals are enhanced with increased iron availability (Weinberg 1974); therefore, another objective of our study was to determine the effect of iron on the virulence of BKD in the salmon. Because it was not known if iron injected into fish would be available for bacterial uptake, radioiron (^{59}Fe) was administered to healthy and BKD-infected fish; the element's distribution into various tissues was then monitored.

Various hematological characteristics were also examined as infection with BKD progressed. This monitoring served two objectives. First, because the determination of LD50 for BKD organisms is not practical, it was desirable to develop a clinical methodology to determine the effects of the disease on the genotypes in the event that no mortalities occurred. Second, we were interested in evaluating the effects of BKD on the general adaptation syndrome which is the nonspecific response of the body to any demand made upon it. Corticoids are generally associated with this system and are known to affect disease resistance in mammals because of their role as immunosuppressants (Selye 1973). We also investigated the dynamics of circulating iron through the progression of the disease.

Materials and Methods

GENOTYPE RESISTANCE STUDY

Yearling coho salmon from the Alsea Salmon Hatchery, Oregon, with mean fork length of 22 cm and weight of 130 g were used as test fish. To determine the transferrin genotype of the fish, we drew approximately 0.5 ml of blood from the caudal vein with 1-ml tuberculin syringes, without seriously injuring the fish. Blood was allowed to clot, and serum obtained by centrifugation was stored at −15°C. A numbered dangler tag was applied below the dorsal fin of each fish for identification. Transferrin genotypes were determined from the serum by using the starch-gel electrophoresis procedure with a discontinuous buffering system described by Ridgway et al. (1970). Five transferrin genotypes, *AA*, *AC*, *CC*, *AB*, and *BC* (Utter et al. 1970) were found in the test fish; but only the first three were used in this study because the *B* allele is not found in the Big Creek stock. The *AA*, *AC*, and *CC* fish were then transferred into separate 70-ℓ fiberglass tanks with flowing, aerated, chilled, dechlorinated water (dissolved oxygen 10.6 mg/ℓ, temperature 9.5 ± 1°C, alkalinity 40 mg/ℓ as $CaCO_3$). Ten fish were held in each tank and fed every other day. The tanks were covered with black polyethylene to help keep the fish quiescent. The fish were acclimated to these conditions for 2 wk before treatment.

The causative agent of BKD used in this study was isolated from infected spring chinook salmon (*O. tshawytscha*) at the Leaburg Hatchery by J. E. Sanders, fish pathologist, Oregon Department of Fish and Wildlife. These cells were cultured on cysteine-enriched Muellar Hinton medium (Difco Laboratories, Detroit, Mich.) until sufficient numbers were obtained for inoculation.

The numbers of fish used in the study are sum-

marized below according to treatment and genotype:

Treatment	Genotype		
	AA	*AC*	*CC*
Saline control	6	6	4
BKD only	20	20	18
Low Fe control	6	6	4
High Fe control	6	6	4
Low Fe + BKD	6	6	6
High Fe + BKD	6	6	6

All fish received two intraperitoneal (i.p.) injections on December 12, 1975. The types of injections administered consisted of the following: 1) saline control fish, two injections of saline, 0.1 and 0.2 ml; 2) BKD only fish, 5×10^5 BKD cells in 0.2 ml saline and a separate injection of 0.1 ml saline; 3) low-iron controls, 28 μg Fe^{+3} (ferric nitrilotriacetate) in 0.1 ml saline and 0.2 ml saline; 4) high-iron controls, 140 μg Fe^{+3} in 0.1 ml saline and 0.2 ml saline; 5) BKD + low-iron fish, 28 μg Fe^{+3} in 0.1 ml saline plus 5×10^5 BKD cells in 0.2 ml saline; and 6) BKD + high-iron fish, 140 μg Fe^{+3} in 0.1 ml saline plus 5×10^5 BKD cells in 0.2 ml saline. To verify infection, gram-stained kidney smears were prepared from all fish that died. The experiment was terminated 60 days postinjection.

Radioiron (^{59}Fe) Distribution Study

Juvenile coho salmon having a mean weight of 55 g were injected i.p. with approximately 1×10^6 BKD cells in 0.1 ml saline; control fish received 0.1 ml saline. Fish were held for 7 days before the ^{59}Fe was administered. Following the 7-day incubation period, each fish was injected i.p. with 0.2 μCi ^{59}Fe-nitrilotriacetate (^{59}Fe-NTA) in 0.2 ml saline. The total iron content of each injection was 140 μg. ^{59}Fe-NTA was prepared by first combining $^{59}FeCl_3$ (5 μCi/μg Fe, Amersham-Searle, Chicago, Ill.) and nonradioactive $FeCl_3$, chelating the iron mixture with NTA, and then raising the pH to 7.4 using sodium bicarbonate. After injection with ^{59}Fe-NTA, we placed the fish in a refrigerated 170-ℓ polyethylene tank having a closed water system and an additional 170-ℓ reservoir. The water was maintained at 52°C, aerated, and recycled through a charcoal filter.

Fish were killed at 4, 8, 24, and 48 h after injection with ^{59}Fe-NTA via a sharp blow to the head. Five experimental and five control fish were used at each sampling period. Fish were exsanguinated by severing the caudal peduncle and blood was collected in heparinized tubes. Whole spleen, liver, and gall bladder, the anterior portion of the kidney, and a section of white muscle located below the dorsal fin and above the lateral line were excised, placed in culture tubes, and weighed. Aliquots of whole blood and plasma were treated similarly. To determine whether or not the ^{59}Fe was excreted from the fish, the body remains of a few individuals were digested in NaOH and samples of charcoal and water weighed. ^{59}Fe content was monitored in each tisue and material using a NaI well counter.

Blood Characteristics Study

Tanks, water conditions, and maintenance of fish were identical to those previously described in the genotype resistance study except that water temperature was 14.5 ± 1.5°C. Each test fish was infected by an i.p. injection of about 1×10^6 BKD cells in 0.1 ml saline. Controls received a 0.1 ml i.p. injection of saline. Groups of fish were killed by a blow to the head at 4, 8. 12, 16, 20, 25, 32, 39, and 46 days after injection. Blood samples were obtained from control groups at days 4 and 46. Blood was collected in heparinized test tubes after severing the caudal peduncle.

We determined hemoglobin levels in 0.02 ml of whole blood by the cyanmethemoglobin method, using Hycel (Houston, Texas) standards and reagents. Packed cell volume was measured by the microhematocrit method. Total plasma protein was monitored with an American Optical refractometer. Blood smears were prepared and stained with Giesma's blood stain. Total plasma iron was determined in 0.2 ml of plasma by the colorimetric procedure described by Caraway (1963). We measured corticoid levels in 0.012 ml plasma using a competitive protein-binding radioassay adapted from Murphy (1967) and the total corticosteroid extraction method outlined by Donaldson and Dye (1975). The binding protein employed was from human male serum, which has a higher affinity for cortisol than cortisone (Slaunwhite and Sandberg 1970). At the time of sacrifice, kidney tissue from all fish was examined for kidney disease bacteria by the Gram-stain technique. Transferrin genotypes of the survivors were determined from the plasma by starch-gel electrophoresis.

Statistical analyses of the data involved either a χ^2 test employing a $2 \times k$ contingency table or a t-test allowing comparison of means with unequal variances and numbers. Comparison of means in the blood characteristics study was accomplished by pooling the data of the two control groups (which did not differ statistically) and comparing these pooled-control values with those of the respective treated groups.

Results and Discussion

A comparison of all dead and surviving infected fish from the iron treated and noniron treated groups demonstrated that the *AA* genotype was the most susceptible, the *AC* had an intermediate susceptibility, and the *CC* was the most refractive to BKD. Approximately twice as many *AA*-fish succumbed to the disease as *AC*-fish, and three times as many *AA*'s died as *CC*'s (Fig. 1A). The difference between the number of *AA*- and *CC*-type fish that died was significant ($P < 0.05$). The results were similar in fish injected only with kidney disease organisms; saline–*AA* fish had the highest number of deaths and *CC* the lowest (Fig. 1B). In the infected, iron-treated fish, mortality was again highest in fish of the *AA* genotype and lowest in the *CC* genotype (Fig. 1C).

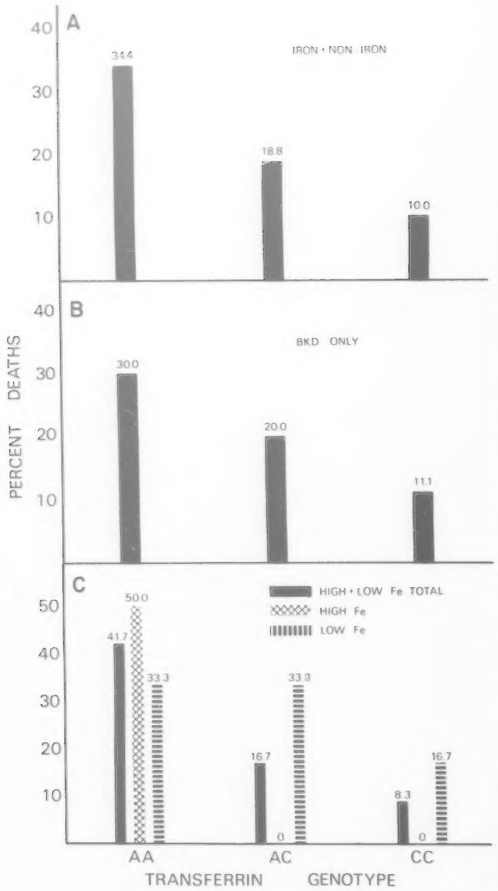

FIG. 1. Percentages of coho salmon (*Oncorhynchus kisutch*) within each transferrin genotype that died of bacterial kidney disease (BKD). A, Pooled data for fish infected with BKD cells and treated or not treated with iron; B, fish injected only with BKD cells and saline; C, after treatment with BKD cells and high and low iron levels.

Iron has been shown to play a key role in the pathology of many bacterial infections by enhancing the growth and virulence of the invading microorganisms (Sussman 1974). Transferrin limits the amount of endogenous iron presented to the pathogens by binding the metal and making it unavailable for bacterial use (Weinberg 1974). The results of our study indicated that fish of the transferrin genotypes examined differ in resistance to BKD. Because of transferrin's bacteriostatic nature in mammals, it is plausible that certain genotypes are better able to compete for iron than others. The mechanism of the differential resistance of fish to BKD could lie in the avidity of the various transferrin proteins for iron. Although transferrin variants in man have not demonstrated significant differences in iron affinity (Giblett 1967), Hershberger (1970) proposed that an increased uptake and release of iron existed in brook trout (*Salvelinus fontinalis*). Increased iron uptake from the plasma and release in storage sites could be a mechanism for resisting invading pathogens which rely on iron for growth and nourishment. Another possible explanation for this difference in resistance between genotypes is the observation that particular phenotypes may have differing overall viabilities. Balakhnin and Galagan (1972) found that the general survival rate of larvae and fingerlings of Ukranian carp (*Cyprinus carpio*) varied according to transferrin phenotype. Therefore, the *CC* fish may have had the highest innate survival rate and the *AA* fish the lowest in our particular study situation. The resistance to BKD may simply be correlated to transferrin genotype but not directly related to the protein. This possibility is further heightened by the fact that the *CC* and *AC* genotypes were approximately 10 times as abundant as the *AA* type in one year-class of coho salmon from the Alsea Hatchery (McIntyre unpublished data).

The *AA* genotype was more susceptible to BKD at high levels of iron than were either the *AC* or *CC* genotypes. Fifty percent of the *AA* fish died, whereas no *AC* or *CC* fish were lost (Fig. 1). Although the *AA* genotype may be less able than other transferrin types to compete with bacteria at high iron levels, the difference may have been fortuitous since only six fish were used in each test group. The combined mortalities of fish treated with high and low iron does closely resemble those in the test group infected with only BKD (Fig. 1). We thus believe that the addition of exogenous iron did not significantly alter the number of deaths in any genotype of coho salmon.

There is considerable controversy concerning the form of iron that should be administered in bacterial enhancement studies. The chelated form of iron used here, ferric NTA, is currently believed to be the form that binds iron to transferrin stoichiometrically and does not create polymeric species like those of certain organic ferric salts (Bates et al. 1967; Hegenauer and Saltman 1975). It is, therefore, plausible that the bacteria in the fish were unable to obtain iron for their own metabolic uses if the iron remained bound to the transferrin protein. However, the results of the radioiron distribution study indicated that the

iron does not remain bound to transferrin. In control and BKD infected fish ^{59}Fe was relatively high in whole blood and plasma 4 and 8 h after injection but gradually decreased over the 24- and 48-h periods. (Fig. 2). This decrease in ^{59}Fe is indicative of the clearance of iron from plasma to other storage sites. Walker (1975) found a similar clearance of ^{59}Fe ($^{59}FeCl_3$) from the plasma of rainbow trout (*Salmo gairdneri*). ^{59}Fe stores in the spleen, kidney, and liver increased with time, while those in gall bladder and white muscle tissue were generally low (Fig. 2). Because of their erythropoietic function, it is understandable why splenic and nephric accumulation of ^{59}Fe occur. However, the liver is also an extremely important site for iron storage in the salmon.

The high affinity of transferrin for iron in the form of Fe-NTA helps explain the consistently higher levels of ^{59}Fe in plasma than in the whole blood. Incorporation of ^{59}Fe into erythrocytes in rainbow trout was found to increase rapidly, reaching a maximum 11 days after treatment with radioiron (Walker 1975). Consequently, the difference in ^{59}Fe content between whole blood and plasma lessens as more radioiron is taken up by the red blood cells.

Levels of ^{59}Fe found in whole blood and plasma are uniformly higher in the BKD-infected fish than in the control fish (Fig. 2). These higher levels are either due to higher transferrin concentrations in the plasma of experimental fish or an otherwise enhanced rate of ^{59}Fe uptake. A correspondingly high level of hepatic ^{59}Fe was also found in the experimental groups, indicating a possible increased rate of iron storage. Infection increased iron stores in the liver of rats by 31% as part of iron-specific nutritional immunity (Pekarek et al. 1972), the process whereby circulating iron levels are decreased so that they cannot be metabolized by pathogens (Kochan 1973). The enhanced transfer of iron to liver stores and the increase of transferrin in the plasma would be advantageous to the host in resisting certain infectious diseases.

Because kidney disease infections are highly resistant to antimicrobial drugs, the results of our study could be significant to fish culturists who are continually plagued by outbreaks of the disease. They could perform specific crosses to maximize resistant genotypes and thus possibly increase hatchery survival and return rates. However, because of the known complexity of genotype–environment interaction and negatively correlated traits, this possibility must be tested by carefully controlled experiments. For instance, although the *CC* genotype was shown to be the

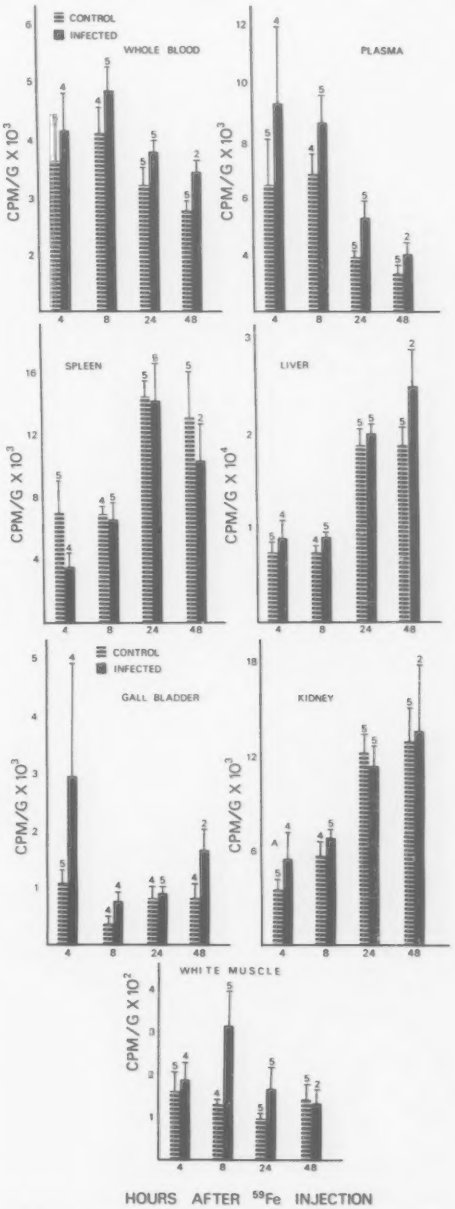

FIG. 2. Mean radioiron activity in whole blood, plasma, spleen, liver, gall bladder, kidney, and white muscle from control and BKD-infected fish at various times following ^{59}Fe injection. Number of fish in each sample is denoted above the bars; the line above each bar represents the mean ±SE.

more resistant to BKD than the *AA* and *AC* genotypes, we have a considerable amount of information (unpublished data) concerning these genotypes in another stock of coho salmon from Big Creek Hatchery that shows the *CC* genotype to be much less frequent than *AA* and *AC*. If the results of our study apply to the Big Creek fish as well as the Alsea fish, resistance to BKD for the *CC* genotype must play a relatively minor role in its overall viability in the Big Creek stock.

The median survival time of fish infected with BKD cells was approximately 40 days; because the blood characteristics of the fish that succumbed to the infection before their scheduled sampling period could not be monitored, the sizes of samples taken between days 20 and 46 were reduced. The resistance to BKD varies from fish to fish, and, although equally challenged, certain fish appeared refractive to the disease and did not manifest any of these symptoms. The "resistant fish" had blood characteristics similar to those of uninfected control fish. The refractiveness of some fish probably increased the mean and standard error for most of the characteristics examined.

Packed cell volume (PCV) and hemoglobin (Hb) concentration apparently decreased with the progression of the disease (Fig. 3). In spite of these decreases, statistically significant differences did not exist between the means for either Hb or PCV in the last group sampled on day 46 (Hb 2.85 g/100 ml, PCV 39.6%). The lack of significant differences may be due to the small size of the test group. If these decreases in PCV and Hb are associated with the infection, they are probably caused by a reduction in erythrocytes or dilution of blood due to water retention. From examination of blood smears, there appeared to be a relative increase of leukocytes and decrease of erythrocytes over the test period. Hemopoiesis in salmonids occurs primarily in the kidney and spleen (Ostroumova 1957). Inasmuch as Wood and Yasutake (1956) noted that both of these organs demonstrated a high degree of morbidity resulting from infection with BKD, the lowering of PCV and Hb may be caused by a slow deterioration of the erythropoietic function in these organs. We found that the kidney was necrotized and the spleen enlarged in nearly all of the highly infected fish. Increase in blood volume, not monitored here, is also a possible source for these findings.

Generally, plasma proteins decreased with progression of the disease (Fig. 3). Most of this protein reduction may have occurred in the albumin fraction of the plasma (Hunn 1964). Because BKD also markedly damages the liver, the synthesis of hepatic protein may have been depressed.

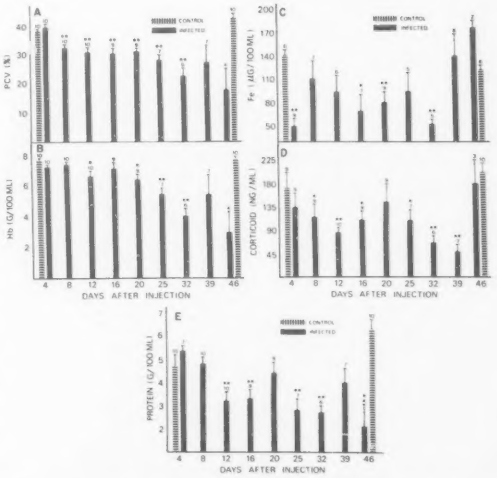

FIG. 3. Mean packed cell volumes (A) and hemoglobin (B), plasma iron (C) plasma corticoid (D), and total plasma protein (E) levels in coho salmon on various days after infection with BKD cells. Number of fish in each sample is denoted above the bars; the line above each bar represents the mean ±SE; $*P < 0.05$, $**P < 0.01$.

Potentially, protein may also have been lost in the urine by nephric failure.

Plasma iron levels were lower in all test groups than in the controls, except on days 39 and 46 (Fig. 3). The mean plasma iron levels in the early and midtest periods ranged from 51.9 ± 11.2 to 110.0 ± 23.8 μg/100 ml. If the pathogens in fish respond similarly to their counterparts in mammals, it may be highly advantageous for the host to limit the availability of endogenous iron presented to invading pathogens. A source of readily available iron is the blood plasma. In mammals, plasma iron has been shown to decrease with the onset of infection (Pekarek et al. 1969). Although such a reduction of iron has not been previously demonstrated in fish, a particularly dramatic decrease in plasma iron was found in the first group sampled 4 days after infection. The mean for this group (51.9 μg/100 ml) was markedly different from the mean for the controls (130.4 μg/100 ml) ($P < 0.01$). The initial response by the fish may have been to sequester iron and relocate it elsewhere. These findings are consistent with the radioiron study; increased transferrin levels in diseased fish may have increased the rate of iron storage in the liver. It is unlikely that blood dilution caused the immediate decrease in the day-4 group because the symptoms of the experimental infection were not yet highly pronounced and diuresis due to handling normally

is of shorter duration. Higher levels of plasma iron in the late test periods may be due to increased hemolysis of blood caused by BKD infection (erythrocytes had reduced integrity in the later stages of the disease and PCV was shown to decrease), the inability of the host's system of iron-specific nutritional immunity — if present in fishes — to function as result of the advanced stages of infection, or the refractory characteristics of fish toward BKD.

The relative amount of corticoids in the plasma appeared to decrease (Fig. 3) as the disease progressed. Six of nine test groups had a mean plasma corticoid level lower than the controls ($P < 0.05$). However, the corticoid levels of the controls were considerably higher than basal levels are believed to be (Schreck unpublished data). Our findings differ from previous research done in mamalian systems where glucocorticoids increase as a result of the general adaptation syndrome of disease-induced stress (Selye 1973). The decrease in plasma corticoids may have been associated with the destruction or exhaustion of the corticoid-secreting interrenal tissue. Upon infection of the head kidney, the ability to produce glucocorticoids may be reduced. If glucocorticoids function as immunosuppressants in fish as they do in mammals, a lack of these hormones could be beneficial to the infected fish.

Transferrin genotypes, number, and percentages of fish used in the blood parameters study are as follows:

Transferrin genotype	No.	%
AA	36	42.9
AC	24	28.6
AB	16	19.0
CC	7	8.3
BC	1	1.2

Of the fish employed in this study, the *AA* genotype was the most prevalent (42.9%) and the *BC* genotype the least (1.2%). Certain fish challenged with BKD organisms were highly refractive and their hematological and histological characteristics were similar to those of the controls. A fish was considered refractive if it had no clinical symptoms of the disease and if hematocrit was 30% or more or if blood hemoglobin was 6.0 g/100 ml (the lowest values found in the controls) or greater. Because the first death attributed to BKD occurred 25 days after infection, only the fish in the test groups on days 25 through 46 were considered for their ability to refract kidney disease. The genotypes of these fish with higher resistance to BKD were as follows: two were *CC*, three were *AC*, and two were *AB*. The absence of the highly prevalent *AA* genotype among the refractive fish and the presence of a relatively high number of *CC* genotype are apparent. These results support the findings of the genotype resistance study that resistance to BKD is correlated to transferrin genotype.

Results from this study indicate that there is a differential resistance to BKD according to transferrin genotype in coho salmon. The *AA* genotype was found to be the most susceptible, the *AC* genotype had an intermediate susceptibility, and the *CC* genotype was the most refractive to the disease. Although radioiron distribution demonstrated that injected iron was probably available to the invading bacteria, exogenous iron did not enhance the pathogenicity of BKD.

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Tissue Accumulation and Enzymatic Effects of Hexavalent Chromium in Rainbow Trout (*Salmo gairdneri*)

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Two-year-old rainbow trout (*Salmo gairdneri*), reared for 2 yr in water containing about 0.00025 mg/ℓ hexavalent chromium (Cr^{+6}) (Naches trout) or between 0.002 and 0.010 mg/ℓ Cr^{+6} (Hanford trout) accumulated appreciable chromium, yielding whole body residues of about 0.029 and 0.18 μg/g wet tissue, respectively. Highest concentrations were in the opercular bone, spleen, kidney, gastrointestinal tract, and gall bladder. Short-term exposure of Hanford trout to 2.5 mg/ℓ Cr^{+6} caused a rapid additional increase of tissue chromium, but at 22 days whole body levels were only 0.87 μg/g. Upon return of exposed fish to water containing 0.002–0.010 mg/ℓ chromium, the metal was rapidly depleted from most tissues except kidney, liver, gill, gall bladder, and bile. Chromium accumulated in tissues of trout exposed to 2.5 mg/ℓ Cr^{+6} was not distributed proportionally among the various subcellular fractions but concentrated in the cell cytosol, especially in the liver and gill.

Mitochondrial cytochrome oxidase, NADH-cytochrome c reductase, and succinate cytochrome c reductase activities in liver, kidney, gill, and brain tissues of Naches trout, Hanford trout, and Hanford trout exposed to 2.5 mg/ℓ Cr^{+6} were not significantly different except for kidney NADH-cytochrome c reductase which was lower in Hanford and chromium treated fish. Microsomal nitroreductase, *O*-demethylase and NADPH-cytochrome c reductase and the soluble glucose-6-phosphate dehydrogenase activities in liver and kidney from Hanford trout were significantly lower than those of Naches trout. Exposure of Hanford trout to 2.5 mg/ℓ Cr^{+6}, however, did not reduce the activities of these enzymes below control levels. In vitro studies showed that trout enzymes were fairly insensitive to Cr^{+6} inhibition. These results suggest that observed differences in enzyme activity between Naches and Hanford trout may be caused by factors other than chromium content of the water.

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Des truites arc-en-ciel (*Salmo gairdneri*) de 2 a., élevées durant 2 a. dans de l'eau contenant environ 0.00025 mg/ℓ de chrome hexavalent (Cr^{+6}) (truites de Naches) ou entre 0.002 et 0.010 mg/ℓ de Cr^{+6} (truites de Hanford) accumulent des quantités appréciables de chrome, donnant pour le corps entier des résidus d'environ 0.029 et 0.18 μg/g de tissu humide respectivement. Les concentrations les plus élevées se rencontrent dans l'os operculaire, la rate, le rein, le tractus gastro-intestinal et la vésicule biliaire. Une courte exposition de truites de Hanford à 2.5 mg/ℓ de Cr^{+6} cause une augmentation supplémentaire rapide de chrome tissulaire. Mais à 22 jours, les niveaux dans le corps entier ne sont que de 0.87 μg/g. Une fois les poissons exposés ramenés à de l'eau contenant 0.002–0.010 mg/ℓ de chrome, le métal disparaît rapidement de la plupart des tissus sauf le rein, le foie, la branchie, la vésicule biliaire et la bile. Le chrome qui s'accumule dans les tissus de truites exposées à 2.5 mg/ℓ de Cr^{+6} n'est pas répartie proportionnellement parmi les diverses fractions subcellulaires, mais il est concentré dans le cytosol cellulaire, surtout dans le foie et la branchie.

Les activités de la cytochrome-oxydase mitochondriale, de la NADH-cytochrome c réductase et de la succinate cytochrome c réductase dans les tissus du foie, du rein, de la branchie et du

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cerveau de truites de Naches, de truites de Hanford et de truites de Hanford exposées à 2.5 mg/ℓ de Cr^{+6} ne diffèrent pas significativement, sauf pour la NADH-cytochrome c réductase du rein, qui est plus basse chez la truite de Hanford et chez les poissons traités au chrome. L'activité de la nitroréductase microsomique, de l'*O*-déméthylase et de la NADPH-cytochrome c réductase, et de la glucose-6-phosphate déshydrogénase soluble dans la foie et dans le rein des truites de Hanford sont nettement plus faibles que chez les truites de Naches. L'exposition de truites de Hanford à 2.5 mg/ℓ de Cr^{+6}, cependant, ne diminue pas l'activité de ces enzymes au-dessous des niveaux des témoins. Des études in vitro démontrent que les enzymes des truites sont assez insensibles à l'inhibition par le Cr^{+6}. Ces résultats donnent à croire que les différences observées dans l'activité enzymique entre les truites de Naches et celles de Hanford peuvent être dues à des facteurs autres que la teneur en chrome de l'eau.

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FISH are relatively insensitive to hexavalent chromium (Cr^{+6}) as the dichromate or chromate anions during acute exposure. Relatively high 24- and 96-h TL_m concentrations of 40–280 and 20–130 mg/ℓ chromium, respectively, have been demonstrated for several species (Trama and Benoit 1960; McKee and Wolf 1963; Pickering and Henderson 1966; Ruesink and Smith 1975). Prolonged exposure, however, increases the toxicity considerably for aquatic organisms. Thus, in studies with young coho salmon (*Oncorhynchus kisutch*), a critical tolerance level of approximately 10 mg/ℓ Cr^{+6} was found following a 14-day exposure period (Holland et al. 1964). In other experiments, Olson and Foster (1956) exposed young chinook salmon (*O. tshawytscha*) and rainbow trout (*Salmo gairdneri*) continuously to various concentrations of sodium dichromate in running water for periods of up to 7 mo. Survival was found to be impaired at 0.080 mg/ℓ and growth was inhibited at only 0.013–0.022 mg/ℓ Cr^{+6}.

Doudoroff and Katz (1953) indicated that Cr^{+6} behaves toxicologically in a manner quite different from most heavy metals. Metallic ions are usually poisonous to fish externally through binding to gill epithelia and interference with respiration. The anionic Cr^{+6}, however, readily penetrates the gill membrane and accumulates in various tissues and organs (Knoll and Fromm 1960).

Direct evidence of an internal site for chromate toxicity was reported by Fromm and Schiffman (1958) who observed severe pathological changes in the intestines immediately posterior to the pyloric ceca in juvenile largemouth bass (*Micropterus salmoides*) exposed to 96 mg/ℓ Cr^{+6}. Gill, the major chromate absorption site (Knoll and Fromm 1960), was unaffected. In rainbow trout, lower environmental Cr^{+6} concentrations (2–4 mg/ℓ) produced a significant increase in hematocrit (Schiffman and Fromm 1959). Also Stokes and Fromm (1965) detected a reduced transport of glucose by cecal and midgut sections from chromium treated rainbow trout. Kuhnert et al. (1976) have recently shown a significant decrease in Na^+, K^+-ATPase of kidney and intestine but not gill and liver in rainbow trout exposed to 2.5 mg/ℓ Cr^{+6}.

The present study was undertaken to achieve further understanding of the sites of chromium accumulation and toxicity in rainbow trout.

Materials and Methods

Two year old rainbow trout (*S. gairdneri*) were used throughout this investigation. One group (Hanford trout), weighing between 250 and 850 g, had been raised in Columbia River water obtained downstream from operating Hanford nuclear reactors which employed once-through cooling. The river water contained 0.002–0.010 mg/ℓ Cr^{+6} (Wooldridge 1968) since sodium dichromate was being added to the cooling water as a corrosion inhibitor (Conley 1954) until these reactors closed down in 1969. The eggs from which this group of fish had been reared were obtained from the State of Washington Department of Fisheries, Naches Fish Hatchery. A second group of fish (Naches trout) weighing from 300 to 800 g, were from the same stock but had been held continuously at the Naches Fish Hatchery in 10°C spring water containing 0.00025 mg/ℓ chromium, presumably all in the hexavalent state (Grogan and Oppenheimer 1955; Cutshall et al. 1966).

Hanford fish were maintained in outside tanks supplied with Columbia River water at approximately 15°C and fed once daily with Clark's New Age trout pellets. Approximately 60 Hanford trout were held for a maximum of 22 days in water containing 2.5 mg/ℓ Cr^{+6} added as $Na_2Cr_2O_7$. Groups of three–six exposed trout were removed at various times throughout the exposure period for chromium analysis or for determination of enzyme activities. Control Hanford trout were treated similarly. At the end of the 22-day Cr^{+6} treatment period, the remainder of the exposed fish were returned to Columbia River water and the loss of tissue chromium followed over an additional 17 days. Naches fish were either killed at the Naches Hatchery for tissue chromium analyses (12 fish) or were transported alive to our laboratory and held in Naches Hatchery water for several days at 10°C in a recirculating chilling unit before being used in enzyme studies (6 fish).

All fish were starved for 24 h prior to killing for tissue chromium analyses or enzyme assays. To determine the subcellular chromium distribution, tissues were quickly removed from groups of six fish, chilled, and the tissues from individual fish homogenized in 2 vol of cold 0.154 M KCl in a Potter–Elvehjem homogenizer. Resulting homogenates were then subjected to differential centrifugation at 600 × *g*, 10,000 × *g*, and 105,000 × *g* for 10, 20, and 60 min, respectively, to prepare the nuclear, mitochondrial, microsomal, and soluble fractions.

For analysis of representative subcellular enzymes, tissues from individual fish were homogenized in 2 vol of cold 0.25 M sucrose containing 0.04 M glycylglycine buffer, pH 7.4. The 600 × *g* supernatant was centrifuged for 20 min at 10,000 × *g* and the resulting supernatant used for the analysis of the microsomal enzymes, nitroreductase, *O*-demethylase, and NADPH-cytochrome c reductase, and for the analysis of soluble glucose-6-phosphate dehydrogenase. The 10,000 × *g* pellet was washed twice by recentrifugation with buffered sucrose and the purified mitochondria used in the analyses of cytochrome oxidase, succinate-cytochrome c reductase, and NADH-cytochrome c reductase.

Enzyme assays were performed as previously described: cytochrome oxidase, succinate-cytochrome c reductase, and NADH-cytochrome c reductase (Caldwell 1969); NADPH-cytochrome c reductase (Buhler and Rasmusson 1968a); glucose-6-phosphate dehydrogenase (Buhler and Benville 1969); nitroreductase (Buhler and Rasmusson 1968a); and phenacetin *O*-demethylase (Buhler and Rasmusson 1968b). Incubations were conducted at 25 C. Protein was assayed by the method of Lowry et al. (1951).

Tissue samples and subcellular fractions for chromium analysis were dried to constant weight at room temperature in vacuo and then ashed at 500°C. Chromium concentration was determined by atomic absorption analysis with a Perkin-Elmer Model 303 atomic absorption spectrophotometer following oxidation of any trivalent chromium in the samples to the hexavalent state with $KMnO_4$ (Feldman et al. 1967).

Tissue concentrations are presented as micrograms chromium per gram wet tissue. To estimate the total chromium residue in certain tissues, the total weight of those tissues was either determined directly or was approximated from the fish weight using percentage factors established for rainbow trout (Giblin and Massaro 1973). Data on total chromium residues in fish tissues have also been normalized to a per kilogram fish basis by a weight correction factor to compensate for the high degree of variability in fish weights among trout of the same age used in these investigations.

Data were analyzed for statistical difference by the standard *t*-test.

Results

Naches trout, raised in water of low Cr^{+6} content (0.00025 mg/ℓ), concentrated this metal in individual tissues from 40- to 4000-fold (Naches control fish, Table 1). Highest chromium levels were found in opercular bone, gall bladder, and anterior kidney. Fish maintained in Columbia River water[1] containing 0.002–0.010 mg/ℓ Cr^{+6} for long periods also had accumulated significant amounts of the metal (Hanford control fish, Table 1); tissue levels ranged from about 5 (opercular bone, brain, skin, and stomach) to 36 (spleen) times greater than those found in the Naches trout. Chromium concentrations were highest in gall bladder, opercular bone, spleen, small intestines, and anterior and posterior kidneys.

When Hanford trout were exposed to 2.5 mg/ℓ Cr^{+6}, most tissues showed only a small additional accumulation of metal in response to about a 1000-fold increase in their environment (Table 1). In most tissues, uptake appeared to be rapid, reaching an equilibrium within approximately 1 day. Tissue concentrations, after chromium exposure, ranged from 1.1 times for gall bladder to 8.5 times for plasma higher than the preexposed fish. With only a few exceptions (gall bladder, bile, anterior and posterior kidney, opercular bone, spleen, and gill), the tissue concentrations of chromium at equilibrium did not notably exceed the Cr^{+6} concentration in the water to which the fish were exposed. Muscle tissue contained substantially lower concentrations of chromium than in the water.

More chromium was associated with skin of Naches and Hanford control fish than with any other tissue (Table 2). Skeletal muscle was secondary in importance. After exposure of the Hanford fish to water containing 2.5 mg/ℓ Cr^{+6}, however, the skeletal muscle became the major storage site for chromium in the fish. Nevertheless, the relative distribution of the metal among most other tissues appeared to be fairly similar over a wide range of chromium exposure concentrations. Whole body chromium residues were 0.029 and 0.18 μg/g for Naches and Hanford trout, respectively. Following exposure of Hanford trout to 2.5 mg/ℓ Cr^{+6}, the total chromium content of the fish rapidly reached an equilibrium, ranging between 0.61 and 0.88 μg/g of the metal over the 22-day exposure period.

When trout initially exposed to 2.5 mg/ℓ Cr^{+6}, were returned to Columbia River water containing only 0.002–0.010 mg/ℓ chromium, a rapid loss of the metal occurred in most tissues (Table 3). Chromium concentrations declined slowly, however, in the gill, liver, gall bladder, bile, and kidney over the 17-day recovery period.

The subcellular distribution of chromium was examined in brain, gill, posterior kidney, and liver tissues of Hanford trout (Table 4). The

[1]Both Naches and Hanford trout were reared on similar commercial pelleted diets containing approximately 70 mg/ℓ chromium.

TABLE 1. Tissue chromium concentration (micrograms per gram wet tissue weight) of Naches and Hanford control rainbow trout (*Salmo gairdneri*) and of Hanford trout following exposure to 2.5 mg/ℓ hexavalent chromium (Cr^{+6}). All values are expressed as means (±SE) and averaged without regard to sex.

Tissue	Controls		Days exposure to Cr^{+6}[b]					
	Naches[a]	Hanford[b]	1	2	4	8	15	22
Opercular bone	1.00 (±0.10)	4.90 (±0.69)	10.3 (±2.5)	6.49 (±1.14)	8.77 (±1.10)	9.12 (±1.15)	9.02 (±0.84)	13.6 (±3.2)
Gall bladder	0.56 (±0.07)	10.6 (±3.2)	5.93 (±1.71)	4.44 (±1.13)	10.9 (±1.9)	2.34 (±0.42)	15.5 (±5.8)	19.1 (±3.7)
Anterior kidney	0.28 (±0.13)	1.71 (±0.01)	4.04 (±0.78)	2.84 (±0.69)	3.96 (±0.48)	5.39 (±0.80)	4.71 (±1.70)	6.43 (±1.20)
Brain	0.22 (±0.08)	1.03 (±0.57)	14.1 (±3.44)	6.19 (±1.87)	5.96 (±1.21)	2.28 (±0.72)	1.49 (±0.29)	3.16 (±1.10)
Vertebral bone	0.21 (±0.20)	0.96 (±0.22)	2.88 (±0.39)	2.54 (±0.59)	2.73 (±0.11)	2.77 (±1.13)	3.21 (±0.27)	3.22 (±0.70)
Bile	0.17 (±0.05)	1.21 (±0.20)	8.31 (±1.68)	4.99 (±1.58)	6.00 (±1.70)	4.10 (±0.69)	3.37 (±1.53)	5.23 (±0.24)
Small intestine	0.17 (±0.03)	1.78 (±0.48)	5.08 (±1.54)	2.21 (±0.27)	4.49 (±1.19)	2.14 (±0.54)	2.54 (±1.27)	4.33 (±0.34)
Colon	0.16 (±0.05)	0.98 (±0.17)	1.14 (±0.20)	1.41 (±0.20)	2.12 (±0.71)	2.19 (±0.42)	1.33 (±0.33)	2.53 (±0.16)
Posterior kidney	0.13 (±0.05)	1.64 (±0.08)	7.09 (±0.90)	7.12 (±1.79)	6.51 (±1.00)	12.2 (±1.3)	7.44 (±2.08)	9.41 (±0.95)
Heart	0.12 (±0.05)	1.58 (±0.48)	2.52 (±0.60)	1.41 (±0.15)	3.90 (±1.04)	2.26 (±0.63)	1.67 (±1.13)	2.06 (±0.40)
Spleen	0.10 (±0.06)	3.57 (±0.55)	6.08 (±1.50)	1.61 (±0.34)	3.38 (±0.98)	4.42 (±1.10)	3.19 (±[illegible]55)	9.46 (±1.94)
Skin	0.095 (±0.03)	0.49 (±0.16)	1.05 (±0.22)	0.81 (±0.16)	1.03 (±0.19)	1.19 (±0.22)	0.96 (±0.27)	2.06 (±0.28)
Fat	0.09 (±0.03)	0.69 (±0.05)	4.58 (±1.75)	3.02 (±0.75)	3.05 (±0.69)	2.05 (±0.40)	1.26 (±0.75)	1.26 (±0.63)
Stomach	0.06 (±0.11)	0.27 (±0.09)	0.91 (±0.13)	0.32 (±0.11)	1.05 (±0.16)	1.55 (±0.26)	1.22 (±0.47)	1.76 (±0.32)
Red cells	0.05 (±0.01)	0.77 (±0.30)	2.54 (±0.68)	2.38 (±0.47)	1.37 (±0.18)	1.64 (±0.17)	1.77 (±0.27)	2.32 (±0.75)
Gill	0.04 (±0.01)	0.65 (±0.11)	5.86 (±0.84)	4.99 (±1.06)	3.70 (±0.56)	6.53 (±0.80)	3.00 (±0.55)	3.27 (±0.25)
Ceca	0.04 (±0.01)	0.43 (±0.13)	1.70 (±0.23)	1.51 (±0.51)	2.42 (±0.38)	2.42 (±0.24)	2.62 (±1.00)	2.16 (±5.62)
Liver	0.04 (±0.01)	0.44 (±0.05)	2.04 (±0.49)	2.61 (±0.73)	1.73 (±0.22)	2.55 (±0.40)	1.53 (±0.31)	2.33 (±0.72)
Plasma	0.03 (±0.01)	0.40 (±0.13)	4.26 (±1.54)	3.14 (±0.39)	3.11 (±0.86)	5.11 (±1.23)	2.50 (±0.42)	2.92 (±0.45)
White muscle	0.01 (±0.00)	0.07 (±0.01)	0.54 (±0.10)	0.29 (±0.09)	0.34 (±0.08)	0.14 (±0.02)	0.31 (±0.08)	0.34 (±0.05)

[a] $N = 12$, mean weight 528 g, range 295–727 g.
[b] $N = 3$ or 6, mean weight = 322 g, range 261–597 g.

chromium content of these tissues increased upon exposure to 2.5 mg/ℓ Cr^{+6} (Table 1). In addition, a greater percentage of the chromium was found in soluble and microsomal fractions from gill and liver, and the soluble fraction of kidney. The percentage chromium in the gill and liver nuclear, liver mitochondrial and kidney microsomal fractions was significantly lower in the fish exposed to 2.5 mg/ℓ Cr^{+6} than in the controls. No differences were found in the subcellular distribution of metal in brain tissue from Hanford trout following exposure to 2.5 mg/ℓ Cr^{+6}.

Activities of three representative mitochondrial enzymes were measured in the same four tissues, i.e. brain, gill, posterior kidney, and liver. With only a single exception, no significant differences were found in the activities of these enzymes from Naches control, Hanford control, and treated fish (Table 5). The activities of succinate-cytochrome c reductase and NADH-cytochrome c reductase were generally lower in the Naches fish than in the Hanford control or treated fish but these differences were not significant ($P > 0.01$) except in the case of NADH-cytochrome c reductase in the posterior kidney.

Mitochondrial enzymes prepared from Naches trout, were generally inhibited in vitro only by Cr^{+6} concentrations higher than those found in the tissues of fish exposed to 2.5 mg/ℓ Cr^{+6}. The order of increasing sensitivity to the metal was: cytochrome oxidase, succinate-cytochrome c reductase, and NADH-cytochrome c reductase (Fig. 1A). Very little inhibition of cytochrome oxidase occurred even at 2×10^{-3} M Cr^{+6}, a concentra-

TABLE 2. Total chromium content (micrograms)[a] of tissues and organs from Naches and Hanford control rainbow trout and in Hanford trout following exposure to 2.5 mg/ℓ hexavalent chromium.

Tissue	Controls		Days exposure to Cr^{+6}[b]					
	Naches[c]	Hanford[b]	1	2	4	8	15	22
Skin[d]	7.86	40.7	88.1	66.9	85.2	85.0	79.7	171
Muscle[d]	5.45	36.3	292	157	183	79.5	167	187
Gastrointestinal tract and contents	4.73	17.3	85.6	106	119	105	94.7	132
Bone[d]	3.30	14.9	44.7	39.3	42.3	42.9	49.7	49.8
Kidney	1.62	14.7	59.4	67.7	54.6	89.2	57.1	75.4
Blood[d]	1.24	17.9	107	86.1	57.8	72.7	68.1	77.2
Gill	1.02	7.66	74.7	63.5	38.2	80.1	37.9	41.4
Fat[d]	0.81	6.03	43.3	39.9	32.1	17.9	10.9	16.9
Liver	0.58	4.48	31.7	33.6	19.4	26.3	16.3	27.3
Other tissues[e]	2.65	23.3	57.6	38.9	36.7	25.9	29.1	93.8
Total	29.3	183	884	699	668	624	610	872

[a]Values corrected to a kilogram fish basis. Total chromium in skin, muscle, bone, blood, and fat was calculated from chromium analyses and an estimate of total tissue mass. The chromium content of all other tissues or organs was measured directly using 8.3% of body weight for skin, 54.5% for muscle, 1.55% for bone, 3.12% for blood, and 0.87% for fat.

[b]$N = 3$ or 6, mean weight 322 g, range 261–597 g.

[c]$N = 12$, mean weight 528 g, range 295–727 g.

[d]Estimated for rainbow trout according to Giblin and Massaro (1973).

[e]Remaining tissue analyzed in this study.

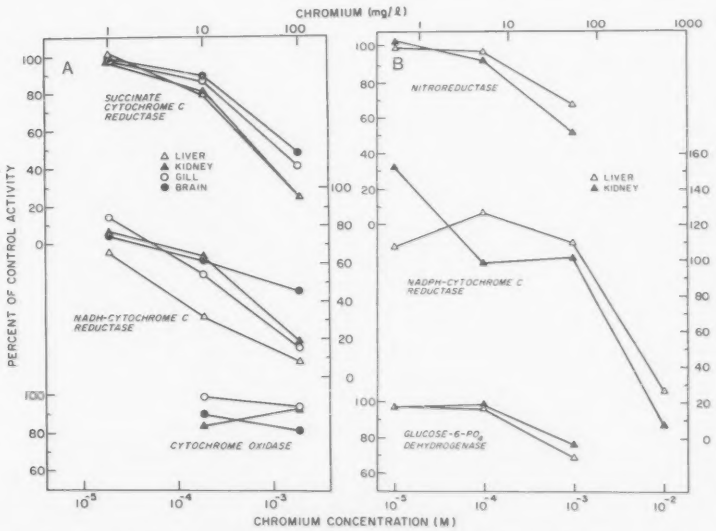

FIG. 1. Effect of hexavalent chromium on the activity in vitro of A, cytochrome oxidase, NADH-cytochrome c reductase, and succinate-cytochrome c reductase in tissues from Naches control rainbow trout (*Salmo gairdneri*). For uninhibited values, refer to Naches control in Table 5; B, glucose-6-phosphate dehydrogenase, NADPH-cytochrome c reductase and nitroreductase in tissues from Naches control rainbow trout. For uninhibited values, refer to Naches control in Table 6.

TABLE 3. Change in tissue chromium concentration (micrograms per gram wet tissue weight) of Hanford trout exposed to 2.5 mg/ℓ Cr^{+6} for 22 days and then transferred to Columbia River water containing 0.002–0.010 mg/ℓ Cr^{+6}. Tissue chromium content expressed as mean (±SE) for three rainbow trout (mean weight 467 g, range 263–733 g). Values averaged without regard to sex.

	Days following transfer from 2.5 mg/l Cr^{+6}					
Tissue	0	1	2	3	5	17
Opercular bone	13.6 (±3.2)	2.80 (±0.40)	3.77 (±0.42)	3.59 (±0.08)	5.38 (±3.20)	2.98 (±0.16)
Gall bladder	19.1 (±3.7)	11.6 (±7.9)	12.7 (±7.7)	6.56 (±0.90)	5.61 (±0.58)	6.92 (±0.73)
Anterior kidney	6.43 (±1.20)	3.82 (±0.34)	7.28 (±0.95)	8.87 (±0.73)	2.20 (±1.06)	5.61 (±1.63)
Brain	3.16 (±1.10)	0.52 (±0.28)	1.77 (±0.36)	1.10 (±0.05)	0.90 (±0.06)	2.71 (±0.89)
Vertebral bone	3.22 (±0.70)	1.22 (±0.13)	1.53 (±0.21)	1.42 (±0.19)	1.37 (±0.11)	1.34 (±0.06)
Bile	5.23 (±0.24)	7.66 (±4.47)	1.57 (±0.95)	3.08 (±1.81)	4.88 (±2.00)	16.3 (±10.1)
Small intestine	4.33 (±0.34)	1.21 (±0.40)	1.34 (±0.27)	1.46 (±0.01)	0.94 (±0.03)	0.66 (±0.24)
Colon	2.53 (±0.16)	1.27 (±0.33)	0.75 (±0.05)	1.15 (±0.07)	0.63 (±0.09)	0.98 (±0.10)
Posterior kidney	9.41 (±0.95)	7.63 (±0.49)	10.5 (±0.9)	12.9 (±1.24)	10.8 (±0.36)	9.33 (±3.14)
Heart	2.06 (±0.40)	0.67 (±0.18)	0.85 (±0.10)	0.75 (±0.02)	0.56 (±0.15)	0.49 (±0.16)
Spleen	9.46 (±1.94)	1.73 (±0.22)	2.75 (±0.31)	2.97 (±0.11)	2.91 (±1.09)	1.55 (±0.03)
Skin	2.06 (±0.28)	1.05 (±0.41)	0.80 (±0.05)	0.79 (±0.15)	0.60 (±0.08)	0.63 (±0.04)
Fat	1.26 (±0.63)	0.99 (±0.62)	0.80 (±0.33)	0.43 (±0.01)	0.59 (±0.33)	0.37 (±0.11)
Stomach	1.76 (±0.32)	0.60 (±0.08)	0.77 (±0.07)	0.77 (±0.02)	0.66 (±0.26)	0.63 (±0.02)
Red cells	2.32 (±0.75)	0.62 (±0.12)	0.92 (±0.13)	0.80 (±0.09)	0.69 (±0.25)	0.55 (±0.08)
Gill	3.27 (±0.25)	2.69 (±0.33)	3.31 (±0.69)	4.17 (±0.19)	3.21 (±1.12)	2.43 (±0.13)
Ceca	2.16 (±5.62)	1.07 (±0.19)	0.96 (±0.20)	0.91 (±0.04)	0.57 (±0.03)	0.54 (±0.09)
Liver	2.33 (±0.72)	1.01 (±0.06)	1.42 (±0.08)	1.54 (±0.30)	0.56 (±0.27)	1.55 (±0.44)
Plasma	2.92 (±0.45)	0.70 (±0.15)	0.84 (±0.22)	0.85 (±0.00)	0.54 (±0.01)	0.49 (±0.08)
White muscle	0.34 (±0.05)	0.08 (±0.02)	0.17 (±0.05)	0.12 (±0.01)	0.05 (±0.02)	0.09 (±0.00)

tion equivalent to 100 μg/g in the tissue. However, succinate-cytochrome c reductase activities were 50–70% inhibited at the same concentration, while those of NADH-cytochrome c reductase were 60–95% inhibited. The latter enzyme was also 50% inhibited at approximately 2×10^{-4} M chromium (10 μg/g), a concentration similar to that found in kidney of Hanford trout exposed to 2.5 mg/ℓ Cr^{+6}. The pattern of inhibition of these mitochondrial enzymes was similar for all tissues studied but the liver enzymes generally were the most sensitive to the metal.

In liver and posterior kidney tissues, the activities of the microsomal enzymes and glucose-6-phosphate dehydrogenase were significantly lower in the Hanford control fish than in the Naches trout (Table 6). Exposure of the Hanford fish to 2.5 mg/ℓ Cr^{+6} for 10 days, however, did not affect the activity of these enzymes. Similar observations were made for nitroreductase activity in gill and brain tissues and for glucose-6-phosphate dehydrogenase activity in gill but not in brain. In the latter tissue, there was no difference in glucose-6-phosphate dehydrogenase activity in

TABLE 4. Percentage distribution of chromium (expressed as mean ± SE) in subcellular fractions of tissues from Hanford control rainbow trout and from Hanford trout after 7 days exposure to 2.5 mg/ℓ hexavalent chromium. Significantly different from control at $*P < 0.005$, $**P < 0.025$, $***P < 0.05$, $\dagger P < 0.10$.

	Hanford[a] control trout	Treated[b] trout
Brain		
Nuclear	30.4±2.2	32.4±3.6
Mitochondrial	23.2±1.6	24.4±3.1
Microsomal	20.4±2.0	19.3±2.3
Soluble	25.9±1.4	25.1±6.6
Gill		
Nuclear	50.3±4.7	28.6±6.7**
Mitochondrial	12.2±1.3	13.6±2.5
Microsomal	7.4±0.4	11.3±1.6**
Soluble	30.1±3.7	46.6±5.6**
Posterior kidney		
Nuclear	37.2±1.1	34.5±4.4
Mitochondrial	14.7±1.1	14.8±1.0
Microsomal	14.0±1.5	10.7±0.8***
Soluble	34.2±2.0	40.1±3.5†
Liver		
Nuclear	47.8±2.7	38.0±3.1**
Mitochondrial	22.1±1.9	12.7±0.9*
Microsomal	11.0±1.5	16.9±1.6**
Soluble	19.1±2.1	32.3±3.1*

[a] $N = 6$, mean weight = 571 g, range 445–730 g.
[b] $N = 6$, mean weight = 516 g, range 436–702 g.

any of the three groups of fish. No *O*-demethylase or NADPH-cytochrome c reductase activities were found in brain or gill tissues of Naches control fish. These two enzymes were found, however, in gill tissues, but not brain, of Hanford control and treated fish. Activities of the two gill enzymes were not significantly different in the two groups of fish.

Inhibition of liver and posterior kidney nitroreductase, NADPH-cytochrome c reductase and glucose-6-phosphate dehydrogenase by Cr^{+6} in vitro is shown in Fig. 1B. The effect of the metal on these enzymes was similar in both tissues. Nitroreductase and glucose-6-phosphate dehydrogenase activities were approximately 40 and 25% inhibited, respectively, at a concentration of 1×10^{-3} M Cr^{+6} but no inhibition of NADPH-cytochrome c reductase was observed at this concentration. At 1×10^{-2} M, however, this latter enzyme was 80–90% inhibited. Essentially no inhibition was noted at 1×10^{-4} M Cr^{+6} (approximately 6 μg/g) for any of the enzymes in either of the tissues studied. The latter concentration is similar to that found in the tissues of fish exposed to 2.5 mg/ℓ chromium (Table 1).

Discussion

Fromm and Stokes (1962) using low concentrations of radio-labeled Na_2CrO_4 showed that 15–20-cm rainbow trout accumulated chromium to levels similar to those present in the water. Thus, maximum whole body concentrations found at 10 days in their trout exposed to 0.0013 and 0.010 μg/g Cr^{+6} were 0.0017 and 0.0134 μg/g chromium, respectively. Fish exposed to Cr^{+6} concentrations > 0.050 mg/ℓ accumulated chromium at a constant rate but failed to reach equilibrium levels during a 28-day exposure period. One group of fish was held in 2.5 mg/ℓ chromium as chromate for 36 days at which time 50% of the fish had died and the chromium in the surviving fish averaged 2.8 μg/g. By contrast, we found in trout exposed to 2.5 mg/ℓ Cr^{+6} that whole body residues after 22 days were only 35% of the exposure concentration. Furthermore, chromium accumulation occurred much more rapidly with maximum levels usually being attained in the first day (Table 2).

Some of the differences between our results and those of Fromm and Stokes (1962) may have resulted from our studies being conducted on 2-yr-old and theirs with yearling fish. The lifetime exposure of our stock of fish to 0.002–0.010 mg/ℓ chromium in the water may also have resulted in an increased capacity for excretion of absorbed chromium. An adaptive response of this type might explain why our fish did not continue to accumulate chromium after the 1st day and why our fish tolerated 2.5 mg/ℓ Cr^{+6} while significant mortalities occurred when Fromm and Stokes (1962) exposed fish to the same concentration.

Rainbow trout that were reared in concentrations of approximately 0.00025 and 0.002–0.010 mg/ℓ Cr^{+6} for periods of up to 2 yr in the present study, accumulated whole body chromium concentrations of 0.029 and 0.18 μg/g, respectively (Table 2). These concentrations were substantially higher than those present in the water to which the fish had been exposed.

Differences in the body burdens observed between short- and long-term chromium exposure suggests two different chromium compartments present in rainbow trout, one with a rapid and one with a much slower rate of exchange. Rapid accumulation of chromium to a concentration similar to that of the exposure water would represent exchange of the fast-turnover pool. The excessive accumulation of chromium by Naches or Hanford stock fish over that present in the water in which they had been reared could result from accumulation within the slow-turnover pool. In support of this possibility, Chipman (1966) has observed two chromium pools in the marine poly-

TABLE 5. Activity of mitochondrial enzymes from Naches and Hanford control rainbow trout and from Hanford trout following 10 days exposure to 2.5 mg/ℓ hexavalent chromium. *$P < 0.01$, significantly different from Naches control.

	Cytochrome oxidase (μmol/[h·g protein])	Succinate-cytochrome[c] reductase (μmol/[min·mg protein])	NADH-cytochrome[c] reductase (μmol/[min·mg protein])
Liver			
Naches control[a]	0.51±0.14	0.10±0.02	0.14±0.04
Hanford control[b]	0.41±0.04	0.06±0.02	0.19±0.01
Treated[c]	0.41±0.05	0.08±0.02	0.21±0.01
Posterior kidney			
Naches control	0.88±0.17	0.16±0.02	0.11±0.02
Hanford control	0.80±0.14	0.21±0.05	0.30±0.03*
Treated	0.78±0.13	0.25±0.04	0.30±0.04
Gill			
Naches control	0.72±0.11	0.04±0.01	0.07±0.01
Hanford control	0.45±0.14	0.08±0.02	0.08±0.02
Treated	0.47±0.14	0.05±0.01	0.14±0.05
Brain			
Naches control	1.07±0.31	0.07±0.03	0.03±0.01
Hanford control	1.01±0.24	0.19±0.03	0.07±0.01
Treated	1.07±0.21	0.16±0.02	0.07±0.01

[a]$N = 5$, mean weight = 644 g, range 478–780 g.
[b]$N = 5$, mean weight = 607 g, range 405–734 g.
[c]$N = 5$, mean weight = 652 g, range 475–850 g.

TABLE 6. Activity of microsomal and soluble enzymes from Naches and Hanford control rainbow trout and from Hanford trout following 10 days exposure to 2.5 mg/ℓ hexavalent chromium. Significantly different from Natches control at *$P < 0.01$, **$P < 0.05$. NA = negligible activity.

	Nitroreductase (μmol/[h·g protein])	*O*-Demethylase (μmol/[h·g protein])	NADPH-cytochrome c reductase (μmol/[min·mg protein])	Glucose-6-phosphate dehydrogenase (μmol/[min·mg protein])
Liver				
Naches control[a]	11.03±0.61	1.66±0.16	0.86±0.16	11.70±1.19
Hanford control[b]	2.73±0.18*	0.19±0.05*	0.25±0.04*	5.49±1.10*
Treated[c]	2.72±0.32	0.15±0.08	0.25±0.02	6.15±0.63
Posterior kidney				
Naches control	10.25±1.86	7.25±1.32	2.13±0.47	11.59±0.42
Hanford control	1.33±0.22*	0.27±0.03*	0.06±0.02*	7.22±1.42**
Treated	2.09±0.32	0.40±0.05	0.29±0.17	8.30±2.61
Gill				
Naches control	1.11±0.11	NA	NA	13.90±2.00
Hanford control	0.11±0.04*	0.11±0.04	0.24±0.07	7.81±0.99**
Treated	0.20±0.08	0.20±0.08	0.20±0.06	9.37±2.63
Brain				
Naches control	2.88±1.21	NA	NA	2.75±0.52
Hanford control	NA	NA	NA	2.39±0.68
Treated	NA	NA	NA	2.18±0.40

[a]$N = 6$, mean weight = 644 g, range 478–780 g.
[b]$N = 5$, mean weight = 607 g, range 405–731 g.
[c]$N = 5$, mean weight = 652 g, range 475–850 g.

chaete worm *Hermione hystrix* with half-lives of 8 and 123 days, respectively. Two chromium compartments have also been shown to be present in the Atlantic croaker (*Micropogon undulatus*) with half-lives of 0.5 and 70 days, respectively (Baptist et al. 1970).

Dichromate and chromate are strong oxidizing agents and Cr^{+6} is, therefore, readily reduced to the Cr^{+3} state in vivo (Feldman 1968). Because of differences in binding behavior of Cr^{+6} and Cr^{+3} in biological systems (Mertz 1969), the rapid- and slow-turnover pools may represent the hexavalent and trivalent forms of the metal, respectively. The rapidly equilibrated pool thus would consist mainly of soluble Cr^{+6} in the cell cytosol and other physiological fluids, whereas the slow-turnover pool would be Cr^{+3} bound to tissue proteins.

A rapid loss of chromium from most Hanford trout tissues occurred upon transfer from high to low chromium water, presumably reflecting the reequilibration of the fast-turnover pool with the ambient chromium concentrations in the water. Chromium tended to remain high, however, in the liver, kidney, gill, gall bladder, and bile, and to a lesser extent, in brain and opercular bone. Chromium is excreted in urine and feces of mammals (Mertz 1969). Similar routes of excretion are probably also present in trout, and as suggested by Knoll and Fromm (1960), the slow loss of chromium from the liver, gill, and kidney may be due to the involvement of these organs in the elimination of metal from the fish. Furthermore, retention of chromium in the liver and high concentrations of the metal in the gall bladder and in bile and intestinal contents provide evidence for the importance of biliary excretion of chromium in the elimination of the metal from trout.

Retention of chromium by certain tissues also could result from differential rates of Cr^{+6} reduction (Feldman 1968) and hence differences in the relative proportion of free Cr^{+6} (fast-turnover compartment) and bound Cr^{+3} (slow-turnover compartment) among tissues. The preferential accumulation of chromium in the cell cytosol of gill and liver of trout exposed to 2.5 mg/ℓ Cr^{+6} provides tentative evidence for the presence of high concentrations of the soluble Cr^{+6} anion in those tissues. In posterior kidney and brain, however, similar proportions of chromium among subcellular fractions before and after Cr^{+6} exposure suggests a higher proportion of Cr^{+3} in those tissues.

Although exposure of Hanford trout to 2.5 mg/ℓ Cr^{+6} resulted in increased amounts of chromium in all subcellular fractions, there were no significant changes as compared with controls in the activities of the representative mitochondrial, microsomal, and soluble enzymes tested (Tables 5 and 6). We also observed that these enzymes were not particularly sensitive to Cr^{+6} in vitro. Only NADH-cytochrome c reductase was substantially inhibited by concentrations of $Cr^{+6} < 10$ mg/ℓ. The latter concentration generally exceeds that found in any of the four tissues of fish exposed to 2.5 mg/ℓ Cr^{+6} with the exception of kidney.

In view of the apparent insensitivity of most of these enzymes to Cr^{+6}, the marked differences in activity of the microsomal and soluble enzymes between the Naches and Hanford control fish are not likely to be a result of chromium inhibition. Nutritional differences between the Naches and Hanford trout could perhaps influence enzyme activity, since there were undoubtedly variations in the frequency of feeding, ration, etc., between the two hatcheries even though the two groups of fish were fed essentially the same diet. Environmental factors such as temperature and water quality may have played an even more important role in producing the observed differences in enzyme activities between the two groups of fish. In particular, the lower temperature of the Naches spring water as compared to that of the Columbia River at the time of the experiment may have resulted in significant differences in enzyme activity. We have found (D. R. Buhler and M. E. Rasmusson unpublished data) for example, that the activities of rainbow trout liver nitroreductase, *O*-demethylase, and glucose-6-phosphate dehydrogenase were 201, 219, and 140%, respectively, higher in Hanford trout acclimated to 5°C than in trout maintained at 18°C.

The possibility remains, however, that these enzymes may be affected by Cr^{+6} only upon very long-term exposure, perhaps by influencing enzyme synthesis. Alternatively, inhibition of the microsomal and soluble enzymes may occur only after reduction of Cr^{+6} to the trivalent state (Feldman 1968). This reduction could be rather slow in trout, thus resulting in gradual accumulation of inhibitory concentrations of Cr^{+3} by the tissues. The results of our accumulation and loss studies lend support to this idea since most chromium remained in the rapid turnover pool, presumably as Cr^{+6}, during the period of time employed in the in vivo enzyme inhibition studies.

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Correlations of Fish Catch and Environmental Factors in the Gulf of Maine[1]

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SUTCLIFFE, W. H. JR., K. DRINKWATER, AND B. S. MUIR. 1977. Correlations of fish catch and environmental factors in the Gulf of Maine. J. Fish. Res. Board Can. 34: 19–30.

In an investigation of catches of 17 commercial marine species of fish and shellfish from the Gulf of Maine, 10 showed statistically significant correlations with sea temperatures at St. Andrews, N.B., or Boothbay Harbour, Maine. Most fish records contained at least 40 yr of data. Descriptive equations are produced for four species based first on the correlation between catch and sea temperature and second on the correlation between catch and sea temperature allowing for fishing effort. Inclusion of fishing effort, not surprisingly, improved the correlations for all of the species so examined. The equations permitted the "prediction" of later parts of the records from earlier parts.

Considering the fish species collectively, the Gulf of Maine system from 1940 to 1959 appeared to be in equilibrium with little fluctuation in the total commercial biomass. We interpret the large fluctuations in individual species abundance as resulting from a combination of fishing pressure and to a significant degree oceanic climate as represented by sea temperature. The small fluctuations in the total biomass displays the species variation, with their differing climatic "preferences," as well as possible predator (including man)–prey relationships. Environmentally imposed patterns underlie at least 50% of the fluctuations in catch of many species and the understanding of these fluctuations is basic to effective management.

SUTCLIFFE, W. H. JR., K. DRINKWATER, AND B. S. MUIR. 1977. Correlations of fish catch and environmental factors in the Gulf of Maine. J. Fish. Res. Board Can. 34: 19–30.

Une étude des prises de 17 espèces commerciales de poissons et de coquillages de mer dans le golfe du Maine démontre pour 10 d'entre elles des corrélations significatives avec les températures de la mer à St. Andrews, N.-B., ou Boothbay Harbour, Maine. Les données pour la plupart des poissons couvrent une période d'au moins 40 a. Nous formulons des équations descriptives pour quatre espèces, fondées d'abord sur la corrélation entre les prises et la température de l'eau, et ensuite en tenant compte de l'effort de pêche. Le fait d'inclure l'effort de pêche améliore les corrélations pour toutes les espèces étudiées, ce qui n'est pas surprenant. Les équations nous ont permis de « prédire » les périodes plus récentes de données à partir des périodes antérieures.

Si l'on considère toutes les espèces de poissons collectivement, on constate que le système du golfe du Maine, entre les années 1940 et 1959, semblait être en équilibre avec très peu de fluctuations dans la biomasse commerciale totale. A notre avis, les grandes fluctuations dans l'abondance d'espèces individuelles seraient le résultat de la pression exercée par la pêche et, à un degré significatif, du climat océanique représenté par la température de l'eau. Les petites fluctuations dans la biomasse totale reflètent la variation des espèces, avec leurs « préférences » climatiques différentes, de même que des relations possibles prédateur (y compris l'homme)–proie. L'environnement est responsable d'au moins 50% des fluctuations dans les prises de ces espèces et qu'il est essentiel pour une gestion efficace de comprendre ces fluctuations.

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IN a preceding paper (Sutcliffe et al. 1976) the role of coastal circulation, especially that of the St. Lawrence River system, on the water properties of the Scotian Shelf and the Gulf of Maine was discussed. That study was initiated when correlations were found between fish catches from these areas and both the environmental factors of local sea temperatures and St. Lawrence River discharge. Evidence was presented to show that events originating within the Gulf of St. Lawrence eventually gave rise to, or were associated with, temperature perturbations on the Scotian Shelf and in the Gulf of Maine. It was thought that the St. Lawrence River system was important, although not necessarily the major influence, in the sequence of events.

With the association between the St. Lawrence River discharge and sea temperatures on the

[1]Bedford Institute of Oceanography Contribution.

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TABLE 1. Data series and sources. New England = N.E.; ICNAF subarea 5 = 5; Nova Scotia and Bay of Fundy = N.S.; Southern New England = S.N.E.

Species	Region	Yr	Source[a]
Alewife	N.E.	1928–71	1–2, 5–9, 12–16
Butterfish	N.E.	1928–71	1–2, 5–9, 12–16
Clams			
Hard	N.E.	1928–71	1–2, 5–9, 12–16
Soft	N.E.	1928–71	1–2, 5–9, 12–16
Atlantic cod	5	1893–1973	11–12
Cusk	N.E.	1928–73	1–2, 5–9, 12–16
Haddock	5	1893–1973	11–12
Hake			
Silver	N.E.	1928–71	1–2, 5–9, 12–16
Red	N.E.	1944–71	1–2, 5–9, 12–16
Atlantic halibut	5	1893–1973	11–12
Atlantic herring	N.E.	1928–71	1–2, 5–9, 12–16
Atlantic mackerel	N.E.	1928–71	1–2, 5–9, 12–16
	N.S.	1893–1964	10
Atlantic menhaden	N.E.	1929–71	1–2, 5–9, 12–16
Pollock	N.E.	1928–71	1–2, 5–9, 12–16
Redfish	5	1936–73	11–12
Sea scallops	N.E.	1928–73	1–2, 5–9, 12–16
Striped bass	N.E.	1930–71	1–2, 5–9, 12–16
Yellowtail flounder	S.N.E.	1935–74	3–4

[a]These follow the bibliographic references at the end of this paper.

Scotian Shelf and in the Gulf of Maine considered, albeit far from understood, we now wish to turn our attention towards the fish. Correlations between the long-term records of commercial fish catch in the Gulf of Maine and sea temperatures and St. Lawrence discharge are presented in an attempt to show the possible effects of the environment on several major fish stocks.

Following the discussion of correlations of individual species with temperature, "predictions" of cod, haddock, and yellowtail flounder catch from temperatures are presented. These consist of estimating one section of the data record based on a relationship derived from another section.

As pointed out in an earlier paper (Sutcliffe et al. 1976), several environmental influences have been considered by a number of investigators with temperature being the most frequently used (e.g. Iselin 1939; Redfield 1939; Carruthers 1951; Templeman and Fleming 1953; Chase 1955; Taylor et al. 1957; Colton and Temple 1961; Martin and Kohler 1965; Flowers and Saila 1972; Sutcliffe 1972; Dickson and Lee 1972; Iles 1973; Gulland 1965; Lett and Kohler 1976). Temperature is not necessarily the most important influence, but it has the best and longest continuous records. Temperature serves only as an indicator of the local oceanographic climate and while the effect may be direct, further investigation is required to determine the actual variable(s) of importance to the fish and its mode of influence.

Methods

Environmental data used here are all mentioned and sources listed in Sutcliffe et al. (1976). These are used with annual commercial catch or landing statistics (Table 1) in correlation analysis almost identical to the method used for correlating environmental data (Sutcliffe et al. 1976).

The annual catch data were first correlated with the mean monthly temperatures of the year corresponding to the year of catch, thus giving 12 correlation coefficients. This was then repeated with the monthly temperature data lagged back a year (e.g. if fish data spanned 1939–73, temperature data was correlated from 1938–72) and so on until an obvious peak (or trough) appeared, to produce a correlation matrix (Table 2). The lag and time of year with the highest coefficient (r) was chosen for presentation. Keeping in mind that much of the environmental effect is in the 1st yr of life (discussed later), the lag was compared with published data on age at commercial size. For all species considered significant, the correlations chosen were not isolated or random events in the matrices (Table 2); several adjacent months or years showed correlations nearly as high. This will be discussed later. Smoothing was done usually by 2- or 3-yr running means and the year shown in the figures is always the 1st yr of the group.

Because a single catch datum for a given year

TABLE 2. Computer generated correlation table of the principle type used in this paper. Files used are monthly mean temperatures for St. Andrews, N.B. and cod, ICNAF subarea 5 (1929–73 used), 3-yr running means. Highest correlation coefficient (r) occurs for August, 4 yr lag. Outline contains values significant at 0.05 level, values underlined significant at 0.02 level, with 6 df. See text.

Yr lag no.	r for mo of											
	1	2	3	4	5	6	7	8	9	10	11	12
0	−.476	−.586	−.557	−.558	−.488	−.443	−.344	−.33	−.347	−.328	−.446	−.426
1	−.545	−.649	−.593	−.647	−.588	−.535	−.514	−.526	−.519	−.5	−.598	−.563
2	−.561	−.653	−.599	−.692	−.677	−.631	−.654	−.682	−.662	−.637	−.714	−.667
3	−.531	−.641	−.611	−.701	−.716	−.678	−.747	−.785	−.738	−.704	−.789	−.718
4	−.453	−.575	−.581	−.678	−.681	−.667	−.796	−.817	−.749	−.74	−.802	−.714
5	−.372	−.501	−.535	−.635	−.613	−.61	−.784	−.791	−.707	−.724	−.746	−.652
6	−.3	−.407	−.471	−.578	−.532	−.535	−.74	−.744	−.659	−.698	−.662	−.57
7	−.252	−.316	−.388	−.493	−.453	−.446	−.677	−.685	−.607	−.651	−.578	−.494
8	−.193	−.204	−.266	−.366	−.335	−.344	−.589	−.622	−.553	−.587	−.502	−.432

usually includes more than one year-class (although often one year-class predominates), the catch data cannot be considered as independent points; for other reasons the same is true for temperature (Sutcliffe et al. 1976). This is further compounded by smoothing. In determining level of statistical significance we have tried to take these factors into account in the same way we handled the environmental data in the previous paper (Sutcliffe et al. 1976). We believe our estimates of significance to be conservative.

In the following presentation most of the fish statistics are compared and correlated with the sea surface temperatures at St. Andrews, N.B. (TEMPSA). This particular environmental data series was chosen because of its time span and geographic location; information was also derived from Boothbay Harbor (BOOTHT) records and a signal representative of the St. Lawrence River system (RIVSUM) for comparison.

Though somewhat distant in space from the actual areas in which the fish have been caught, the sea surface temperature trends at St. Andrews have been shown to be representative of surface and subsurface temperature variations through the Scotian Shelf and the Gulf of Maine (see Lauzier 1972). For those fish statistic records beginning prior to St. Andrews sea temperatures, correlations were run with sea temperatures at Boothbay Harbor, Maine, or air temperatures at Eastport, Maine. Comparisons between Eastport air and St. Andrews sea temperatures can be found in Sutcliffe et al. (1976).

In choosing catch records for analysis and presentation, few particualr criteria were observed other than the necessity that a given record be for one species only, the data derived from a fairly limited area (in the ecological sense) and contain a reasonably long series. Convenience also influenced our choices. For example, while the fisheries statistics of Nova Scotia are available for many years, these include catches inside the Gulf of St. Lawrence and outside on the Shelf, quite different habitats in our opinion, and would require much sorting out of landing data by fisheries districts of the province. Fisheries of New Brunswick presented the same problem. Thus, confining our attention to the area of the Shelf from Cape Breton, N.S., to a little past Cape Cod, Mass., our presentation is mostly centered in the New England fisheries (from Fisheries Statistics of the United States) and International Commission for the Northwest Atlantic Fisheries (ICNAF) subarea 5 (from ICNAF publications).

Results and Discussion

The following species taken individually were run through our programs and are presented as they occurred. We did not attempt to go into each in any great detail for reasons given later. Some have already been noted or indications given in the literature that stocks are susceptible to environmental fluctuations (e.g. alewife, Leim and Scott 1966; striped bass, Merriman 1941) so we do not pretend originality in this respect; we have merely updated them and added longer records (Fig. 1).

ALEWIFE (*Alosa pseudoharengus*)

Although frequently spawning in fresh water near the sea (Leim and Scott 1966), stocks of this anadromous species appear to be related to sea temperature. Figure 1A shows the New England catch from 1928 to 1971 and temperatures for the month of April with a lag of 6 yr ($r = 0.850$). In 1972 commercial catches were mostly 2–8 yr old (ICNAF 1973), dominated by ages 4 and 5 (5th and 6th growing season).

BUTTERFISH (*Peprilus triacanthus*)

Figure 1B shows the New England commercial catch of butterfish from 1928 to 1971 with St. Andrews temperatures (April and May) lagged by 4 yr ($r = 0.735$). From information in Bigelow and Schroeder (1953), the lag may be a little long although a 3-yr lag gave $r = 0.717$. Slightly higher correlation coefficients (but not significant) were observed for October–November and lags of 4–5 yr.

ATLANTIC COD (*Gadus morhua*)

Landings from ICNAF subarea 5 (from Coté 1952; Martin and Kohler 1965; and subsequent

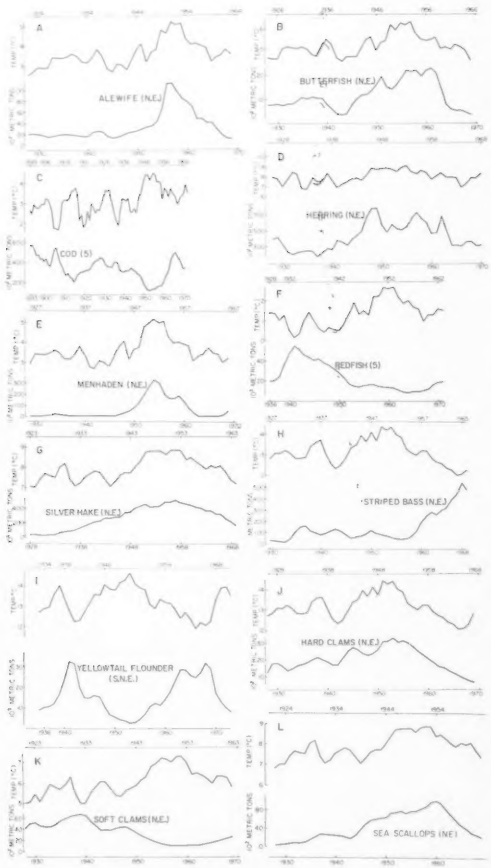

FIG. 1. Commercial fish landings and St. Andrews sea temperatures (except Fig. 1C). All are 3-yr running means except where indicated. For further details see Table 3. A, Alewife (*Alosa pseudoharengus*), New England; B, butterfish (*Peprilus triacanthus*), New England; C, Atlantic cod (*Gadus morhua*), ICNAF subarea 5 (3-yr means) and July–August Eastport air temperature (4-yr means); D, Atlantic herring (*Clupea harengus harengus*), New England; 2-yr running means; E, Atlantic menhaden (*Brevoortia tyrannus*); New England; F, redfish (*Sebastes marinus*), ICNAF subarea 5; G, silver hake (*Merluccius bilinearis*), New England; H, striped bass (*Morone saxatilis*), New England; I, yellowtail flounder (*Limanda ferruginea*), southern New England, 2-yr running means; J, hard clam (*Mercenaria mercenaria*) (meats), New England; K, soft-shell clam (*Mya arenaria*) (meats), New England; L, Sea scallop (*Placopecten magellanicus*) (meats), New England.

ICNAF Stat. Publ.) were plotted with Eastport air temperatures from 1893 to 1973. The results (Fig. 1C) have a correlation coefficient of −0.661. With St. Andrews surface temperatures (1921–73) and a lag of 4 yr, r assumed a value of −0.833 for July and August temperatures. In the 1920s most cod on Nantucket Shoals were 3–5 yr old (Schroeder 1930) as were those on Georges Bank 1956–70 (Brown and Heyerdahl 1972).

ATLANTIC HERRING (*Clupea harengus harengus*)

Plots of New England herring and temperature (November) with a 2-yr lag ($r = 0.604$) are shown in Fig. 1D. The lag time is short for adult fish in the commercial catch (ages 4 and 5, Boyar 1968); however, the New England landings include the inshore fishery for immature fish so the lag derived for the mixture given here may not be unreasonable. Clearly, any further work on these correlations should separate stocks and the fisheries.

ATLANTIC MENHADEN (*Brevoortia tyrannus*)

New England menhaden landings are correlated with St. Andrews sea temperatures (April, $r = 0.872$) with a 3-yr lag (Fig. 1E). Henry (1971) indicates the average age of specimens from the North Atlantic fishery (north of 40°) was 3.7 yr from 1955 to 1968.

REDFISH (*Sebastes marinus*)

Kelly and Wolf (1959) give the age composition for redfish in the Gulf of Maine for the early 1950s. The spread is wide, ranging from about 3 to over 20 yr. However, the "mean" age of 8 yr selected in our lagging program does not seem unlikely when looking at the Kelly and Wolf histograms. Redfish catch from subarea 5 and St. Andrews temperatures are correlated in Fig. 1F ($r = -0.728$). Data previous to 1936 were ignored because the fishery had not yet developed (E. Sandeman personal communication).

SILVER HAKE (*Merluccius bilinearis*)

The 5-yr lag found for the New England catch of silver hake (see Fig. 1G $r = 0.781$) is close according to the age estimate given by ICNAF (1964) for this species (3–4 yr, 4th–5th growing season). Correlations from 1928 to the early 1960s showed a lag of 4 yr.

STRIPED BASS (*Morone saxatilis*)

New England catches (mostly Massachusetts and Connecticut) and August temperatures ($r = -0.631$) for a 3-yr lag are shown in Fig. 1H. According to Merriman (1941) 2-yr-olds (entering or in the 3rd growing season) appear most prominently in the commercial catch.

Yellowtail Flounder (*Limanda ferruginea*)

Brown and Hennemuth (1971) have described the yellowtail flounder fisheries in subarea 5. We have used their tabulated data for the southern New England grounds and updated the catch and effort from Edwards and Hennemuth (1975). Correlating the catch from this stock with St. Andrews temperature, we obtained the highest negative correlation (−0.792) for August with a 2-yr lag. The data are plotted in Fig. 1I. Sissenwine (1974) also found strong correlations with temperature for this species.

Hard Clams (*Mercenaria mercenaria*)

The 2-yr lag for commercial catch of this species (Fig. 1J) seems reasonable ($r = 0.805$). Resently, most of the hard clams harvested are near the lower size limit in Maine (5.1 cm), according to E. S. Gilfillan (personal communication), and approximate 2-yr-olds.

Soft-Shell Clams (*Mya arenaria*)

A 7 yr lag and negative correlation ($r = -0.798$) were found between New England clam catch and St. Andrews temperatures (November and December) (Fig. 1K) with a secondary peak for a 5-yr lag with February and March temperatures ($r = -0.745$). Minimum size restrictions place limits at 5.1-cm shell size in Maine (the largest producer of the area), an average age of 5 yr (Dow and Wallace 1957), although growth rate is highly variable.

Sea Scallops (*Placopecten magellanicus*)

Dow (1964a) indicated the correspondence between spring and autumn sea temperatures (Boothbay Harbor) and Maine landings (1939–43) of this species. He noted that 6–9-yr-olds composed most of the catch in the late 1940s and 1950s.

Correlating New England scallop catches for 1928–71 with St. Andrews temperatures we obtained the highest correlation (0.796) for November with a lag of 6 yr (Fig. 1L). For Georges Bank catches (Caddy and Lord 1971) and St. Andrews temperature, the highest correlation (0.821) was for March and a lag of 8 yr, which is too long (J. F. Caddy personal communication). However, the character of the fishery changed considerably during the 1960s, perhaps contributing to the discrepancy (Bourne 1964; Caddy and Lord 1971).

Other Species

In addition to the foregoing, several other species in our area of interest have been correlated with temperature. Lobster (*Homarus americanus*) abundance–temperature correlations were shown by Flowers and Saila (1972), and indications given for northern shrimp (*Pandalus borealis*) and the blood worm (*Glycera dibranchiata*) by Dow (1964a, b). With Atlantic mackerel (*Scomber scombrus*), Taylor et al. (1957) showed positive correlations between New England landings and New Haven air temperatures (1810–1930) for a 3-yr lag.

Attempts were made to use Atlantic halibut records (*Hippoglossus hippoglossus*) for subarea 5 (1893–1973, ICNAF Sta. Bull.) which were compared with appropriate Eastport air temperatures. While significant at the 0.05 level (Table 3) we are inclined to question the statistical value of the low levels of catch (mean < 500 t/yr) and the table was somewhat confusing, perhaps because of the considerable spread in age found in the catches (McCracken 1958). For red hake (*Urophycis chuss*) correlations with Boothbay Harbor temperatures did not fit an easily recognizable pattern, although with St. Andrews and RIVSUM results were obtained with a 2-yr lag (not inconsistent with 2–3 yr age, Rickter 1972) (see Table 3). Efforts to derive useful associations from landings of cusk (*Brosme brosme*) and pollock (*Pollachius virens*) were not fruitful.

Other Environmental Records

Most of the species presented above for correlations with St. Andrews sea temperatures were tried also with Boothbay Harbor temperature records and RIVSUM. Table 3 gives the results of these and the levels of significance with all three signals. With one signal or another, 13 species showed significant (0.05 level or less) relations with the environmental records. There are some differences in year lags between Boothbay and St. Andrews series, but the significance of these is doubtful. In many cases, although the highest coefficient was chosen for presentation, neighboring ones in terms of adjacent months or years were almost as high and probably not significantly different statistically.

We are reluctant to put great importance on the particular month indicated in Table 3 for the highest coefficient. It is more likely that a span of months is involved, as encountered when going into cod, haddock, yellowtail flounder, and menhaden in greater detail (see below), and that this span (representing the first growing season?) is possibly more important biologically to the species in question.

Gulland (1953, 1965) has advised caution with the use of correlations to relate environment to

TABLE 3. Statistical data for some of the correlations of environmental factors and catches of various species. Environmental signal = Env. Sig.; Running mean = RM; New England = N.E.; St. Andrews, N.B. = S.A.; Boothbay Harbour, Me. = B.H.; RIVSUM = R; ICNAF subarea 5 = 5 (under "area"); Eastport, Me., air temperatures = EPATEM; Southern New England = S.N.E.

Species	Area	Yr	Env. Sig.	RM	Mo for Env. Sig.	Yr Lag	r	No. of ind. points[a] Fish	Env. Sig.	df[a]	Significance
Alewife	N.E.	1928–71	S.A.	3×3	4	6	.850	8	10	6	.01–.001
		1928–71	B.H.	3×3	2	5	.841	8	8	6	.01
					12	7	.867	8	6	4	.05–.02
		1928–71	R	3×3	4	6	.850	8	14	6	.01–.001
Butterfish	N.E.	1928–71	S.A.	3×3	4, 5	4	.735	8	11	6	.05–.02
				3×3	10	5	.750	8	10	6	.05–.02
		1928–71	R	3×3	3	5	.788	8	9	6	.02
		1928–71	B.H.	3×3	4	5	.786	8	9	6	.02
Clams											
Hard	N.E.	1928–71	B.H.	3×3	10	3	.744	7	8	5	.05
		1928–71	R	3×3	4	2	.696	7	14	5	.1–.05
		1928–71	S.A.	3×3	8	2	.805	7	10	5	.05–.02
Soft		1928–71	S.A.	3×3	11, 12	7	−.798	6	8	4	.1–.05
				3×3	2, 3	5	−.745	6	9	4	.1–.05
		1928–71	R	3×3	1	5	−.800	6	9	4	.05
		1928–71	B.H.	3×3	11	7	−.886	6	6	4	.02
Atlantic cod	5	1914–73	B.H.	3×3	10	4	−.668	9	12	7	.05
		1925–73	S.A.	3×3	7–12	4	−.850	7	8	5	.02–.01
		1919–73	R	3×3	3	4	−.778	8	10	6	.02
		1925–73	S.A.	3×3	7, 8	4	−.833	7	10	5	.02
		1893–1973	EPATEM	3×4	10–12	4	−.661	12	25	10	.02–.01
Cusk	N.E.	1928–71	S.A.	3×3	12	7	−.543	7	10	5	> .1
		1928–71	B.H.	3×3	12	7	−.680	7	6	4	> .1
		1928–71	R	3×3	12	1	−.731	7	10	5	.1–.05
Haddock	5	1893–1923	EPATEM	3×3	11–2	2	−.548	7	12	5	> .1
Hake											
Silver	N.E.	1928–71	S.A.	3×3	11	5	.781	5	9	3	> .1
		1928–71	R	3×3	3	6	.771	5	9	3	> .1
		1928–71	B.H.	3×3	11	4	.880	5	6	3	.05
Red	N.E.	1944–71	B.H.	3×3	8	0	.522	12	6	4	> .1
		1944–71	S.A.	3×3	8	2	.507	12	7	5	> .1
		1944–71	R	3×3	6	2	.847	12	8	6	.01
Atlantic halibut	5	1893–1973	EPATEM	3×3	3	11	−.537	17	39	15	.05–.02
Atlantic herring	N.E.	1928–71	B.H.	2×2	11	2	.737	10	8	6	.05–.02
		1928–71	R	2×2	3	4	.648	10	12	8	.05–.02
		1928–71	S.A.	2×2	11	2	.604	10	10	8	.1–.05
Atlantic menhaden	N.E.	1929–71	S.A.	3×3	4	3	.872	7	11	5	.01
		1929–71	R	3×3	3	3	.796	7	9	5	.05–.02
		1929–71	B.H.	3×3	2	2	.881	7	8	5	.01–.001
					12	3	.870	7	6	4	.05–.02
Pollock	N.E.	1928–71	S.A.	3×3	8	2	.463	8	10	6	> .1
		1928–71	B.H.	3×3	8	2	.458	8	11	6	> .1
		1928–71	R	3×3	12	2	−.453	8	11	6	> .1
Redfish	5	1936–73	B.H.	3×3	11	8	−.846	6	5	3	.1–.05
		1936–73	S.A.	3×3	3	8	−.728	6	9	4	.1
		1936–73	R	3×3	2	8	−.867	6	6	4	.05–.02
				3×3	12	10	−.861	6	7	4	.05–.02
Sea scallops	N.E.	1928–71	R	3×3	3	7	.820	6	9	4	.05
		1928–71	S.A.	3×3	11	6	.796	6	8	4	.06
		1928–71	B.H.	3×3	11	5	.885	6	10	4	.02
Striped bass	N.E.	1930–71	B.H.	2×3	7	3	−.644	6	9	4	> .1
		1930–71	S.A.	2×3	8	3	−.631	6	9	4	> .1
		1930–71	R	2×3	5	5	−.763	6	18	4	.1–.05
Yellowtail flounder	S.N.E.	1935–74	S.A.	2×2	8	2	−.792	10	12	8	.007
		1935–74	B.H.	2×2	8	1	−.797	10	12	8	.007
		1935–74	R	2×2	4	2	−.586	10	17	8	.08

[a]For method of determining independent points and degrees of freedom see Sutcliffe et al. (1976).

fluctuations in populations because of the chance of spurious relations. Some presented here may be fortuitous. Redfish is certainly a possibility as is silver hake with only one main peak of catch correlated with one main peak in temperature; only more years of data with further and detailed study will tell. The choice of variables — four different environmental signals, month, year lag, and running average — is not as great as would appear. In a previous paper (Sutcliffe et al. 1976) we reviewed the great similarity among a number of signals along the coast (including the ones used here) and it is not surprising that the monthly components would show basic similarities. The fact that the year lags we found are not greatly dissimilar to the likely mean ages of the various species at commercial size since the 1920s supports our thesis since strength of year-class is thought to be largely determined in the 1st yr of life (Gulland 1965; Templeman 1972; Cushing

TABLE 4. Data and statistical information for species considered with effort (see text). ICNAF subarea 5 = (5); Southern New England = (S.N.E.); North Atlantic = (N.A.). y = catch (10^3t), x_1 = temperature, x_2 = effort, days fished ($\times 10^3$).

Fish	Equation	Yr RM	Period	Constants			
				a	b	c	r
Atlantic cod (5)	$y = a + bx_1$	3	1925–71	107.55	−7.3792	—	.850
(Brown and Heyerdahl 1972)	$y = a + bx_1 + cx_2$	3	1931–71	117.83	−9.8425	.82792	.896
	"	1		118.79	−9.6235	.45425	.833
Haddock (5)	"	3	1917–68	69.599	−6.2417	4.4543	.776
(Grosslein and Hennemuth 1973)	"	1		64.579	−4.3132	3.6125	.751
Yellowtail flounder (S.N.E.)	$y = a + bx_1$	2	1935–74	147.79	−11.436	—	.811
(Brown and Hennemuth 1971; Edwards and Hennemuth 1975)	$y = a + bx_1 + cx_2$	2	1937–74	132.89	−10.441	1.3832	.882
	"	1	1937–74	76.539	−5.9172	2.0516	.828
Atlantic menhaden (N.A.) (Henry 1971)	"	1	1941–68	−107.13	12.19	.05663	.924

1974; Daan 1975). The method of running the correlations is an averaging process in terms of year lag. Actually, using earlier or later segments of some of the records, we find somewhat longer or shorter lags indicating catch composition of older or younger fish. As to running means, increased smoothing results in greater correlation coefficients indicating the importance of low frequencies and long-term trends. This, however, decreases statistical significance; less smoothing increases significance and decreases the magnitude of r.

With some of the species presented in Table 3, lags with RIVSUM are longer than those of the temperature records. This is consistent with the notion presented in the earlier paper that the St. Lawrence River system has some influence further south months later. Again, however, it is difficult to assign significance to the actual difference in year lags or months involved between RIVSUM and the other two records for reasons given above.

RECORDS WITH EFFORT DATA

Results so far have dealt only with "raw" catch or landing statistics and make no attempt to take into account changes in fishing effort in terms of time or equipment expended. Four records that included considerable time series of standardized effort statistics were examined in more detail – cod, haddock (*Melanogrammus aeglefinus*), yellowtail flounder, and menhaden (see Table 4).

Figure 2A (upper part) shows the cod catch of ICNAF subarea 5 from 1925 to 1973. For the period 1925–50 an equation was derived from annual data (least squares fit) of the form $y = a + bx$ from the relationship between cod (y) and St. Andrews sea temperatures (x_1), $r = 0.640$. The second part of the middle graph (Fig. 2A) extending to 1971 was "predicted" from this equation. The occurrences of major peaks and troughs are reasonable except for the reduced relief of the fitted curves and a 2-yr difference of the peak in the late 1960s ($r = 0.850$). At the

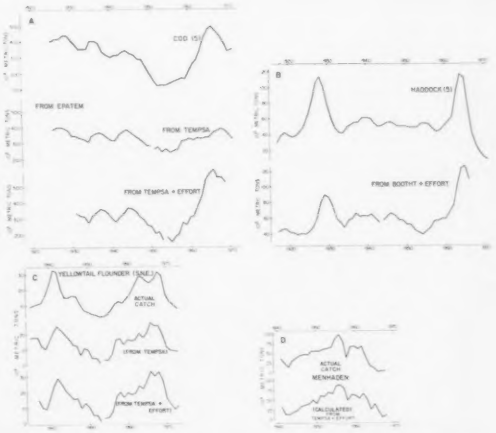

FIG. 2. A, *Upper* — ICNAF Subarea 5 cod catch; *middle* — calculated catch from temperature record (St. Andrews sea July–December); *lower* — calculated catch from sea temperature and effort. All 3-yr running means. B, *Upper* — ICNAF subarea 5 haddock catch; *lower* — catch calculated from temperature (Boothbay January–August) and effort. All 3-yr running means. C, *Upper* — southern New England yellowtail flounder catch; *middle* and *lower* — calculated from temperature (St. Andrews June–October) and effort. All 2-yr running means. D, *Upper* — annual North Atlantic menhaden catch; *lower* — annual catch calculated from temperature (St. Andrews May–December) and effort.

bottom of Fig. 2A is a graph of catch (y) derived from $y = a + bx_1 + cx_2$ (x_1 = temperature St. Andrews, x_2 = days fished $\times 10^{-3}$) for 1931–55. From this the remainder was calculated to the end of the effort record (1971). Similarities (and differences) with the actual catch record are obvious. The correlation coefficient of the entire cod–temperature–effort relationship is 0.896 (see Table 4).

With haddock, Eastport air temperatures for the early part of the record (starting in 1893) gave indifferent confusing results, explaining only

about 25% (r^2) of the variation, and failed completely to account for the considerable rise in catch in the late 1920s. A large influence on this peak was greatly increased effort (Hennemuth 1969; Grosslein and Hennemuth 1973). So, using Boothbay temperatures (x_1), effort (x_2), and annual catch (y), we fitted the equation $y = a + bx_1 + cx_2$ for 1917–43 and extrapolated catch for the remainder using only temperature and effort (10^{-3} days). The results are shown in Fig. 2B (3-yr means); the correlation coefficient for the entire relationship was 0.776 (see Table 4).

The same approach used for cod was employed for yellowtail flounder (southern New England). The results (Fig. 2C) show, top, catch from 1935 to 1972; middle, catch and temperature fitted for 1935–53, catch predicted from the first period by temperature 1954–74; and bottom, catch fitted with temperature and effort (10^{-3} days) for 1937–53 to predict catch for 1954–74, all 2-yr running means. Details for catch–temperature and catch–temperature–effort equations for the entire series are given in Table 4.

Figure 2D shows plots of (upper) annual catch of North Atlantic menhaden and (lower) calculated catch from St. Andrews temperatures and standard vessel days, $r = 0.924$ (Table 4). For temperature alone, $r = 0.760$. The use of running means apparently did not introduce any spurious correlations in the other three species discussed above as seen in Table 4 when annual catch was used.

We realize the equations we have used here are not the usual ones found in mathematical treatments of fish stocks. Catch and effort might ideally have a curvilinear relationship and the relations to temperature might more properly be some function of the reciprocal of the absolute temperature. Clearly, if effort declines to zero, so must the catch, whereas once effort exceeds some optimal value, the catch should eventually go down. However, for the ranges of effort and temperature encountered here, the linear function did nearly as well as (or a little better than) more complicated ones that were tried and no purpose seemed to be served by making the presentation more complex. With these empirically derived equations we wish to make no further point than that environment should be considered when dealing with these populations.

No attempt was made to extend these catch–temperature–effort relationships with quite recent data for two reasons: 1) we feel the best catch and effort data available for a species result from a single investigator or group that has spent considerable time evaluating and collecting the data for that species, and 2) we are unsure of the effect of quotas on recent data. Ideally, some of the calculations presented here should be redone for the catch of a single country for which all the factors are well known.

Without more attention to the details of the biology and life histories of the various species considered here, it is difficult to say much about the underlying causes for positive or negative correlations. For instance Welch (Anon. 1975) notes that a negative correlation for soft-shell clams is likely through the agency of a predaceous crab that is more plentiful in warm years. Except for striped bass all species of fin fish showing negative correlations in this report are near the southern limit of commercial abundance (Bigelow and Schroeder 1953; Leim and Scott 1966).

Periodicity

If, as this study suggests, trends in the catches of commercial fisheries are associated with trends in the sea temperatures then any periodic behavior in the latter becomes important in forecasting future oscillations. Such an occurrence was recently discussed by Southward et al. (1975), who observed an 11-yr cycle in the commercial catches of several species of fish off England. They related this perodicity to sunspot cycles through sea temperatures.

To investigate the periodicity within the environmental factors of this study autocorrelations and power spectra were run on the annual averages of the sea temperatures at Boothbay Harbor (1906–73) and St. Andrews (1921–73), the air temperatures at Eastport (1874–1972) and the St. Lawrence River discharge, RIVSUM (1914–73). In each case the autocorrelation showed similar curves to that of Boothbay Harbor (Fig. 3) with a peak occurring at a lag of around 22 yr. The lag at which the peak occurs, the correlation coefficient and its significance follows:

	r at peak	Yr lag at peak	P
Sea surface temp.			
Boothbay Harbor	.261	22	.10
St. Andrews	.180	22	.10
Air temp			
Eastport	.366	22	.10
River discharge			
RIVSUM	.373	26	.08

Significance was calculated in a manner similar to that found in Sutcliffe et al. (1976).

Power spectra of these same series showed, after trend removal, that most of the variance is explained by fluctuations on the order of 20 yr or greater. More variance, although still quite small, was observed at higher frequencies in the

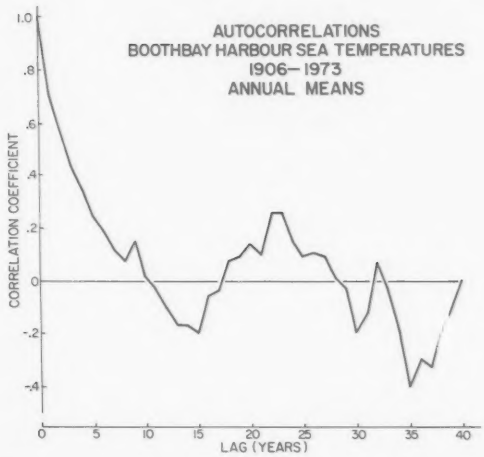

FIG. 3. Autocorrelations, Boothbay Harbor sea temperatures, see text.

air temperatures at Eastport than in the remaining series.

Although the environmental data series are too short to establish the stability of a 22-yr periodicity it is interesting that other investigators have also noted such a period, e.g. Roberts and Olsen (1975) for the occurrence of droughts in the Great Plains of North America.

SUBAREA 5

Figure 4 shows 3-yr running means of catch for the various species discussed above for subarea 5. ICNAF statistics were used back to 1954 (except for cod, haddock, and redfish for which the records were longer) and before this the New England landings (less Connecticut) were used. For a few years in the 1950s, before the influx of large mobile fleets, the numbers agreed fairly well so this combination of the two records does not seem unreasonable; for 1953–56, according to ICNAF records, the U.S. catch was at least 99% of the total in subarea 5, and presumably before this. In spite of wide variations of individual species and many factors affecting the data such as economic influences, the total is curiously flat for many years not unlike Lake Erie (Regier and Hartman 1973). While this is a log plot which would tend to flatten relief, the extremes are only about ±10% of the mean for the period of this plot. The picture from about 1940 to 1959 suggests a balanced system with man as an opportunistic predator taking whatever was in greatest abundance but with a relatively steady supply in toto. After 1960 the effect of large fleets with increased effort may be seen (discussed by Edwards (1968) and Brown et al. 1973). According to these authors the new level of catch constitutes overfishing of the major stocks involved.

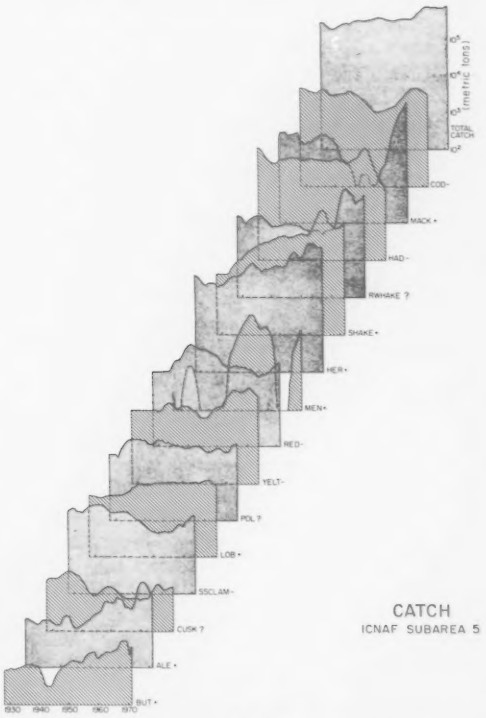

FIG. 4. ICNAF subarea 5 "catch" of various species (round fresh) and total (at top), see text, 3-yr running means,

Most of the species depicted in Fig. 4 have been shown above to undergo fluctuations correlated with environmental changes and the entire roster amounts to an average of about 84% of the total catch for the area. Further, the correlations with the environmental signals, in the majority of cases, explain at least 50% (r^2) of the variation. Clearly, a better understanding of the basic patterns of fluctuations, apparently imposed in part by the environment, would help in management policies and the imposition of quotas.

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Added in proof: We wish to cite an independent but parallel investigation which has been brought to our attention (Dow personal communication). Although the approach differs somewhat, the primary conclusions agree with ours: R. L. Dow. 1977. Effects of climatic changes on the relative abundance and availability of commercial marine and estuarine species. J. Cons. Cons. Int. Explor. Mer 37(3). (In press)

Development of Atlantic Salmon (*Salmo salar*) Eggs and Alevins Under Varied Temperature Regimes

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PETERSON, R. H., H. C. E. SPINNEY, AND A. SREEDHARAN. 1977. Development of Atlantic salmon (*Salmo salar*) eggs and alevins under varied temperature regimes. J. Fish. Res. Board Can. 34: 31–43.

Atlantic salmon (*Salmo salar*) eggs and alevins were raised under conditions where the temperature was systematically varied either at fertilization, at the eyed egg stage, or at hatching. Mortality was more than 20% in eggs started immediately after fertilization at constant incubation temperatures <4°C as compared with 5% or less at temperatures >4°C. Alevins that eyed at 8°C and higher were progressively smaller the higher the temperature. The optimum temperature from fertilization to eye pigmentation was near 6°C. Eyed eggs reared at lower temperatures until hatching were larger than those hatched at higher temperatures. This size differential was maintained until the yolk was completely absorbed at all posthatching temperatures investigated. Sudden decreases in temperature at the eyed egg and hatching stages induced severe edema of the alevin yolk sac, resulting in slower growth and increased mortality.

PETERSON, R. H., H. C. E. SPINNEY, AND A. SREEDHARAN. 1977. Development of Atlantic salmon (*Salmo salar*) eggs and alevins under varied temperature regimes. J. Fish. Res. Board Can. 34: 31–43.

Des œufs et des alevins de saumon atlantique (*Salmo salar*) ont été élevés dans des conditions de température systématiquement variées soit au moment de la fécondation, au stade d'œuf embryonné ou à l'éclosion. Les mortalités dépassent 20% pour des œufs soumis immédiatement après la fécondation à des températures d'incubation constantes <4°C, comparativement à 5% ou moins à des températures >4°C. Les alevins soumis à une température de 8°C ou plus au stade d'œuf embryonné sont progressivement plus petits à des températures de plus en plus élevées. La température optimale entre la fécondation et la pigmentation de l'œil se rapproche de 6°C. Les œufs embryonnés élevés à de plus basses températures jusqu'à l'éclosion sont plus gros que ceux élevés à de plus hautes températures. Cette différence dans la taille se maintient jusqu'à ce que le vitellus soit complètement résorbé à toutes les températures d'après éclosion étudiées. Des abaissements soudains de température au stade d'œuf embryonné et à l'éclosion provoquent un grave œdème du sac vitellin de l'alevin, causant un ralentissement de la croissance et une augmentation de la mortalité.

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THE influence of temperature upon the development of salmonid eggs and alevins has been the object of experimentation for over 100 yr since Ainsworth (1859, reported by Embody 1934) determined times from fertilization to hatching of brook trout (*Salvelinus fontinalis*) eggs for a series to temperatures from 2.8–12.2°C.

Salmonid eggs can tolerate a temperature range of 0–16°C (Embody 1934; Hokanson et al. 1973; Hayes et al. 1953; Seymour 1956 from Alderdice personal communication); however, little has been published on the effects of temperature variation from fertilization through embryonic development to posthatching stages.

This study investigates some of the consequences of systematic temperature variation on the development of Atlantic salmon (*Salmo salar*) eggs and alevins. Specific aspects considered are the effects of temperature variation on egg and alevin mortality, time from fertilization to first observed development of eye pigmentation, time from eye pigmentation to hatching, embryonic length at hatching, rate of growth from hatching to complete yolk utilization, and size at completion of yolk absorption. Some of these aspects will be discussed in relation to the practice by many culturists of using cumulative temperature–time data (i.e. degree–days) to assess development.

Materials and Methods

ORIGIN OF EGGS

The experiments were performed during the winters of 1973–74 (experiment 1) and 1974–75 (experi-

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ment 2). In experiment 1 all eggs were the progeny of one pair of salmon netted from Dennis Stream, a 5.4-kg female and a 8.1-kg male. The fish were stripped, and the eggs fertilized at the St. Andrews Biological Station, water hardened, then placed in hatching boxes. Eggs for experiment 2 were of Miramichi River stock, obtained from the South Esk fish hatchery at South Nelson, N.B. They arrived at the St. Andrews laboratory 5 h after fertilization and were placed in the hatching boxes upon arrival.

Experimental Conditions

The rectangular hatching boxes (20 × 10 × 4 cm) were painted black to exclude light. Water flowed into one end of the boxes through a 0.7-cm ID (inside diameter) tube centered 0.75 cm from the bottom and out of the other end through a 0.9-cm ID tube centered 0.9 cm from the top. Plexiglass shelves, 1.5 cm from the bottom, were glued to each end of each box. The bottoms of the boxes were covered with a layer of washed pebbles, previously dried at 105°C. Over the gravel, eight 0.7-cm OD (outside diameter) glass tubes were set into sockets in the plexiglass shelves. The tubes were arranged in four pairs, the tubes of each pair close enough to support a single row of eggs. About 50 eggs (mean of 54, range of 40–63) were counted into each box. Mean flow rates through the boxes were 122 ml/min (range 50–254) in experiment 1 and 103 ml/min (30–300) in experiment 2. Mean velocity of water movement past the eggs was 3.0–4.0 cm/min. Oxygen determinations were not made in experiment 1, but weekly determinations were made in experiment 2 (unmodified Winkler). Oxygen concentrations, determined from the outflow, were always above 90% air saturation.

Dechlorinated water was passed through two titanium coils submerged in an antifreeze bath to cool the water to the lowest experimental temperature. The water was then warmed as it passed through a cascading system of compartments with the water raised 2°C in each compartment. Water of the appropriate temperature was tapped to supply the boxes. Water in all compartments of the heating system was vigorously aerated.

The boxes were placed on six shelves of an aluminum rack, with all boxes on a shelf at a common temperature. When the temperature for a particular group of eggs was to be changed, the box was moved to the appropriate shelf. The water temperature in each box was noted twice daily by inserting a thermistor into the box via the outflow tube.

The eggs were dosed weekly with 1/200,000 malachite green for 15 min by adding the prophylaxis to the water supply.

Experimental Design

Experiment 1 — Eleven boxes of eggs were placed at each of six temperatures, 2, 4, 6, 8, 10, and 12°C (Fig. 1). When 100% of the eggs had developed eye pigmentation at a particular temperature, one box of eggs was placed at each of the other five temperatures. The remaining six boxes continued at the initial temperature. Similarly, when 50% of the eggs were hatched in the remaining six boxes, one box was placed at each of the other five temperatures. Thus, one box of eggs from each or the original 11 remained throughout development at its initial temperature. In all, there were 66 groups of eggs, with each group experiencing a unique combination of temperatures from fertilization to first development of eye pigmentation (T_1), from eye pigmentation ("eyeing") to 50% hatching (T_2), and after hatching (T_3). The term incubation temperature is used to refer to constant temperature from fertilization to complete yolk absorption.

Experiment 2 — Lids of black plastic cloth were taped over the boxes in experiment 1. These proved to be unsatisfactory because older alevins pushed them up and escaped. Consequently, much information about alevin development after hatching was lost.

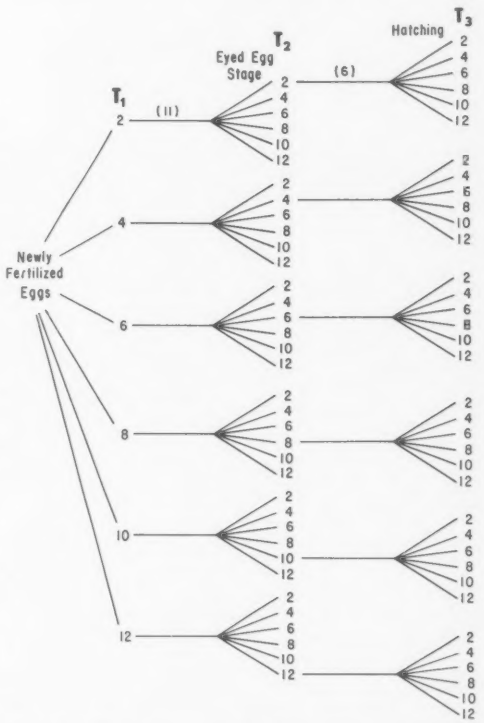

FIG. 1. Flow diagram for design of experiment 1. Newly fertilized eggs divided into 66 lots, 11 lots at each temperature (T_1). At 100% eyed stage, 5 lots from each of the original 11 were placed one at each of the other temperatures; the remaining 6 lots were held at T_1. Temperatures from eyed stage to hatching are designated T_2. At 50% hatch, the remaining 6 lots again were subdivided, 1 lot to each temperature. Posthatching temperature = T_3.

TABLE 1. Mean experimental temperatures for each experimental group in experiment 1. The target temperature regimes are listed above each line (e.g. 2–2–2 is the temperature regime for the group nominally held at 2 °C from fertilization through to free-swimming). The top number in each column is the mean temperature from fertilization to development of eye pigment (i.e. 2.6 for the 2–2–2 group); the middle number is the mean temperature from "eyeing" to hatching; and the bottom number is the mean temperature after hatching (i.e. 2.5 for the 2–2–2 group).

2–2–2	2–2–4	2–2–6	2–2–8	2–2–10	2–2–12	2–4–4	2–6–6	2–8–8	2–10–10	2–12–12
2.6	3.5	2.6	2.7	3.0	2.8	2.7	3.1	3.2	3.3	3.7
2.3	2.3	2.0	2.5	3.0	2.9	4.0	6.1	7.8	9.1	11.8
2.5	3.8	6.1	8.6	10.1	11.5	4.0	6.3	8.4	10.1	11.8
4–4–4	4–4–2	4–4–6	4–4–8	4–4–10	4–4–12	4–2–2	4–6–6	4–8–8	4–10–10	4–12–12
4.0	4.5	4.3	3.8	3.9	3.5	4.0	4.3	4.8	5.0	4.5
3.8	3.9	3.8	3.7	3.7	3.6	2.9	6.0	7.6	9.4	11.1
4.0	2.3	6.2	8.3	9.9	11.2	2.7	6.0	7.9	9.5	11.6
6–6–6	6–6–2	6–6–4	6–6–8	6–6–10	6–6–12	6–2–2	6–4–4	6–8–8	6–10–10	6–12–12
5.8	6.2	6.0	5.9	5.8	5.7	5.7	5.8	6.1	6.1	6.2
5.9	5.9	5.9	6.0	6.0	5.9	2.4	4.0	7.5	9.2	11.6
6.1	2.6	4.2	8.0	9.6	11.7	2.1	4.1	7.9	9.6	11.7
8–8–8	8–8–2	8–8–4	8–8–6	8–8–10	8–8–12	8–2–2	8–4–4	8–6–6	8–10–10	8–12–12
8.0	7.9	7.8	7.7	7.8	8.0	7.3	7.5	7.5	8.2	8.2
7.7	7.7	7.7	7.9	7.6	7.6	2.7	3.2	5.9	9.7	11.5
7.9	2.3	4.2	6.1	9.4	11.3	2.5	3.8	5.8	9.6	11.6
10–10–10	10–10–2	10–10–4	10–10–6	10–10–8	10–10–12	10–2–2	10–4–4	10–6–6	10–8–8	10–12–12
10.0	9.6	9.4	9.5	9.6	9.6	9.5	9.6	9.4	9.5	10.0
10.0	9.8	9.9	9.5	9.6	9.6	2.5	3.4	6.1	7.7	11.8
9.8	2.7	4.1	6.2	7.6	11.8	2.2	3.6	6.0	7.7	11.9
12–12–12	12–12–2	12–12–4	12–12–6	12–12–8	12–12–10	12–2–2	12–4–4	12–6–6	12–8–8	12–10–10
11.7	11.5	11.5	11.6	11.7	11.7	11.7	11.8	11.8	11.5	11.6
11.6	11.6	11.6	11.5	11.4	11.7	2.4	3.6	5.8	8.0	9.7
11.7	2.5	4.1	6.3	7.6	9.6	2.0	3.9	5.8	7.4	9.4

Therefore, tight fitting plexiglass lids were used in experiment 2, which was performed to better examine the influence of temperature on posthatching development. In addition, some of the tests of experiment 1 on development to hatching were repeated. Thirty-five boxes of about 50 eggs each were incubated at five constant temperatures, 4, 6, 8, 10, and 12°C (seven boxes at each temperature).

MEASUREMENT OF ALEVIN LENGTH

Alevin length was measured to the nearest 0.5 mm (tip of nose to midpoint of outer margin of tail). The measurement corresponds to fork length in older alevins where the tail had noticeably forked. Alevins were anesthetized in MS-222® (ethyl *m*-aminobenzoate methanesulfonate) prior to measurement. No deaths attributable to use of anesthetic occurred.

In experiment 1, samples of 10 alevins from each box were measured within 24 h of hatching, as follows. As soon as hatching had begun in a given box, 12 eggs were put in a plastic petri dish and replaced in the hatching box. The eggs in the petri dish were inspected twice daily for newly hatched alevins, which were removed and measured. In addition to measurement of newly hatched alevins in each box, usually two measurements were made on samples of 10 alevins from each box at irregular intervals after hatching. All alevins were returned to their original boxes after measurement.

In experiment 2, samples of at least 10 alevins from one of the seven boxes at each temperature were measured within 24 h of hatching. In two other boxes at each temperature, samples of 10 alevins were measured at regular intervals after the median hatching date. Different intervals were used at different temperatures to compensate for different rates of development. These intervals were 5, 6, 7, 8, and 9 days for temperatures of 12, 10, 8, 6, and 4°C, respectively. We were concerned about the possibility that the frequent length measurements might affect alevin growth. Therefore, alevins in another two boxes from each temperature were measured only half as often (e.g. at 10-day intervals at 12°C). As a control, alevins from the two remaining boxes at each temperature were measured only at completion of yolk absorption. All alevins were returned to their respective boxes after measurement.

TEMPERATURES

The mean temperatures for experiment 1 (Table 1) and 2 (Table 2) are within 0.5°C of the "target" temperatures (2, 4, 6, 8, 10, 12) except where 2°C was required in experiment 1. Early in experiment 1, it proved difficult to maintain temperatures near 2°C, so that some temperatures in which some of these lots of eggs were held during the period from fertilization to development of eye pigmentation are nearer 3°C than 2°C. The standard deviations are

TABLE 2. Mean temperatures at various stages of development in experiment 2; fert. = fertilization.

Nominal temp	Temp (fert. to "eyeing")	("eyeing" to hatching)	(post-hatching)
4	4.7	4.0	4.0
	4.6	4.0	4.0
	4.7	4.0	4.0
	4.6	4.0	4.0
	4.6	4.0	4.0
	4.5	4.0	4.0
	4.7	4.0	4.0
6	6.0	5.5	6.0
	6.0	5.6	6.0
	6.0	5.4	6.0
	6.0	5.4	6.0
	6.0	5.4	6.0
	6.0	5.5	6.0
	6.0	5.5	6.0
8	7.8	7.5	7.7
	7.7	7.5	7.7
	7.9	7.7	7.8
	7.7	7.6	7.7
	7.7	7.5	7.7
	7.8	7.6	7.7
	7.8	7.3	7.8
10	9.8	10.0	9.2
	9.8	10.0	9.2
	9.8	10.0	9.2
	9.8	10.0	9.1
	9.9	10.0	9.2
	9.8	10.0	9.2
	9.9	10.0	9.2
12	11.1	11.9	11.6
	11.1	12.0	11.7
	11.0	11.8	11.6
	11.0	11.9	11.6
	11.0	11.8	11.6
	11.1	12.0	11.6
	11.1	12.0	11.7

0.5°C or less for all temperatures. The data are analyzed in relation to the actual mean temperatures in Tables 1 and 2. For ease of presentation, however, the "target" temperatures are referred to in the text. In addition, the temperature regime of any given group from fertilization to development of eye pigmentation will be termed T_1, that from development of eye pigmentation to hatching as T_2, and that from hatching to the end of the yolk absorption at T_3. In experiment 2, temperatures were constant throughout ($T_1 = T_2 = T_3$).

Results

Egg and Alevin Mortality

Egg mortality in experiment 1, expressed as a percentage of the eggs originally placed in the boxes, was minimal at T_1 = 4–8°C (Table 3, row *a*). Mean egg mortality within this temperature range was 5%. Mortality for eggs held at $T_1 < 4$°C was 22%, and mean egg mortalities at T_1 = 10 and 12°C were 13 and 11%, respectively. Some mortality at T_1 = 10–12°C may have resulted from fungal infection. Mortality at T_1 = 2°C was not due to fungus. Egg mortality in Table 3 includes unfertilized eggs, because dead eggs were not examined for evidence of development.

Six groups of eggs were kept at constant temperature until hatching (i.e. $T_1 = T_2$). Mortality at T_1, T_2 = 2°C was significantly greater than mortalities at all other temperatures ($P < 0.005$, Duncan's multiple-range test and analysis of variance, percent mortality subjected to arc sine transformation before statistical analysis). Mortality at T_1, T_2 = 10–12°C was significantly higher than at 4, 6, and 8°C in 2 of 10 comparisons.

Egg mortalities for all lots with T_1 = 2°C, but with T_2 varying from 4 to 12°C, were pooled and tested against those lots for which $T_1 = T_2 = 2$°C (i.e. Table 3, row *a* for T_1 = 2°C, first six entries tested against the last five). The difference was not significant (Student's *t*-test), nor were the differences significant for similar tests on egg mortalities in the other *a* rows of Table 3. Therefore, high egg mortality for all lots at T_1, T_2 = 2°C is attributable specifically to the low T_1.

As in experiment 1, egg mortality was high at 12°C (Table 3 and 4) in experiment 2, again possibly because of fungal infection.

Alevin mortality in experiment 1 (Table 3, row *b*) averaged 2.5% (range 0–10%) for most groups. Excluded from this mean were data from groups in which the temperature was decreased from 12 to 2 or 4°C at eye pigmentation or at hatching. Alevin mortality for these latter groups averaged 14%. Such temperature decreases resulted in other complications to be discussed later.

High alevin mortality (mean of 32%) occurred at 12°C in experiment 2 (Table 4). In contrast, no alevin mortality occurred in the one group maintained at 12°C ($T_1 = T_2 = T_3$) in experiment 1. The reason for the high mortality in experiment 2 is unknown.

Development of Eye Pigmentation vs. Temperature

Time required for development of eye pigmentation varied inversely with temperature (Fig. 2). The eggs in experiment 2 (of Miramichi origin) developed eye pigment more quickly than those in experiment 1.

TABLE 3. Experiment 1. Egg and yolk-sac alevin mortalities at 66 experimental temperature regimes. The first temperature (Celsius) in each triplet (e.g. 2–2–2) is the temperature for the period from fertilization to eyeing (T_1), the second from eyeing to hatching (T_2), and the third during posthatching development (T_3). *a*, percent mortality prior to hatching, *b*, percent posthatching mortality.

	2–2–2	2–2–4	2–2–6	2–2–8	2–2–10	2–2–12	2–4–4	2–6–6	2–8–8	2–10–10	2–12–12
a	16	21	27	19	31	16	8	19	15	27	25
b	2	4	0	0	4	4	6	6	6	6	0
	4–4–2	4–4–4	4–4–6	4–4–8	4–4–10	4–4–12	4–2–2	4–6–6	4–8–8	4–10–10	4–12–12
a	4	1	4	4	8	4	6	11	6	4	4
b	0	4	0	0	2	0	0	7	7	2	0
	6–6–2	6–6–4	6–6–6	6–6–8	6–6–10	6–6–12	6–2–2	6–4–4	6–8–8	6–10–10	6–12–12
a	4	2	6	5	5	7	2	0	2	7	6
b	0	8	0	4	2	0	0	10	2	4	0
	8–8–2	8–8–4	8–8–6	8–8–8	8–8–10	8–8–12	8–2–2	8–4–4	8–6–6	8–10–10	8–12–12
a	4	2	6	5	8	3	4	4	7	9	6
b	0	8	0	0	0	3	0	4	8	4	0
	10–10–2	10–10–4	10–10–6	10–10–8	10–10–10	10–10–12	10–2–2	10–4–4	10–6–6	10–8–8	10–12–12
a	15	10	7	6	18	6	19	6	25	25	14
b	7	8	0	0	0	0	6	4	2	0	0
	12–12–2	12–12–4	12–12–6	12–12–8	12–12–10	12–12–12	12–2–2	12–4–4	12–6–6	12–8–8	12–10–10
a	16	16	4	8	8	11	16	16	21	10	0
b	16	0	5	4	0	0	12	16	0	0	0

TABLE 4. Experiment 2. Temperature was constant from fertilization throughout development. *a*, percent mortality prior to hatching; *b*, percent posthatching mortality.

	4	6	8	10	12
a	2.2 (0–4)	8.6 (4–14)	5.6 (0–16)	7.2 (2–12)	14.4 (4–28)
b	1.4 (0–4)	2.0 (0–8)	2.0 (0–4)	4.2 (0–14)	32.2 (12–50)

ALEVIN SIZE

Experiment 1 — Alevin lengths at hatching for the various combinations of T_1 and T_2 are presented in Table 5. Inspection of the data indicates that T_1 has a lesser effect on alevin length at hatching than does T_2. Alevin length at hatching progressively decreases as T_1 is raised above 6°C (for any given T_2). Alevin length is independent of T_1 at <6°C. Length at hatching is a linear function of T_2 for a constant T_1, with length decreasing roughly ⅛ mm for each degree increase in temperature. Lengths approach 21 mm at $T_1 = T_2 = 2$°C, and decrease to <17 mm at $T_1 = T_2 = 12$°C.

When a regression surface is fitted to the data of Table 5, using a quadratic polynomial as a model, the equation $l = 21.82 - 0.0079\ T_1^2 - 0.3279\ T_2$ provided an adequate fit, where l = alevin length at hatching, T_1 = eyeing temperature, T_2 = hatching temperature. Analysis of variance indicated that the T_1, T_2^2, and T_1T_2 terms of the second degree polynomial could be omitted without seriously affecting the goodness of fit. The partial correlation coefficient (r) for the T_2 term is 0.95 and the multiple R is 0.96.

Experiment 2 — Alevins from eggs of Miramichi origin were smaller at hatching for all temperatures than were Dennis alevins at equivalent temperatures (Table 6). Although egg diameters were not measured, the smaller size of Miramichi alevins at hatching possibly is associated with smaller egg size.

TIME FROM EYEING TO HATCHING

Experiment 1 — Time from eyeing to hatching was affected much more by T_2 than by T_1 (Table 5). For a given T_2, T_1 has < 10 days influence on time from eye pigmentation to hatching over the range of temperatures tested. The Q_{10} of the reciprocal of the time, as a function of T_2, is about 8 for the range of 2–12°C. At T_1 below 8°C, eyeing-to-hatching times were maximal and nearly constant for a given T_2. At $T_1 > 8$°C, hatching time decreased with increasing temperature.

When a regression surface is fitted to the data, the quadratic equation, $t = 119.27 - 0.070\ T_1^2 - 17.62\ T_2 + 0.775\ T_2^2$ was found adequate (t = time from development of eye pigment to hatching). R is 0.97 and the regression accounted for a

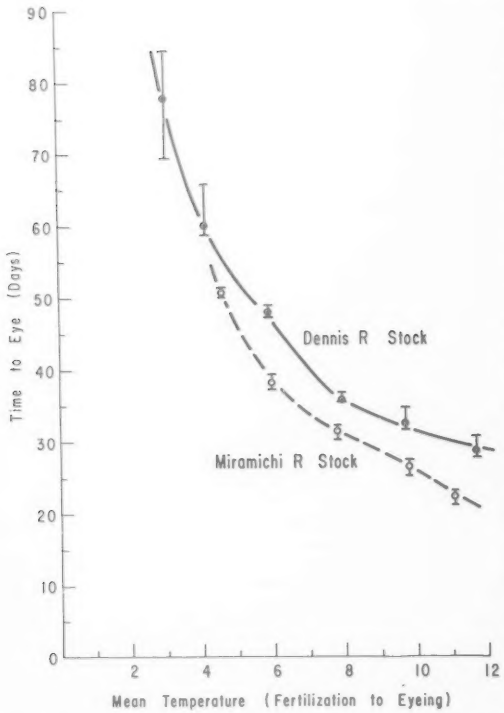

FIG. 2. The time for appearance of eye pigmentation at various temperatures for Atlantic salmon (*Salmo salar*) eggs of Miramichi and Dennis stocks. Each point represents the mean of 11 groups of 50 eggs each for Dennis stock and 7 groups of 50 eggs each for Miramichi stock. Bars indicate ranges.

significant portion of the treatment variance ($P < 0.001$).

The responses of length at hatching and of time from development of eye pigmentation to hatching with respect to T_1 and T_2 are quite similar. If a semilogarithmic transformation had been performed on times from eye pigmentation to hatching with respect to T_2, then the response surface would have been similar to that relating alevin length to T_1 and T_2. Consequently, the data can be combined (Fig. 3) where the relation between T_2 and log time from eye pigmentation to hatching is linear. The equation is $\log t = 2.13 - 0.094\ T_2$. This line can be dissected into segments corresponding to length at hatching. At higher T_2 (and shorter times from eye pigmentation to hatching), smaller alevins are produced at hatching. Variation in T_1 adds little error, since T_1 affects length at hatching and time from eye pigmentation to hatching in the same way.

The relationship shown in Fig. 3 can explain some of the variability in times from eye pigmentation to hatching. For example, at $T_1T_2 = 6$, 2°C, the median time was 90 days — longer than that predicted by the response surface equation. Alevin length at hatching in this group was also greater than expected, so that the point is not out of line with the rest of the data of Fig. 3. The explanation appears to be that the eggs ($T_1T_2 = 6$, 2°C) were past full term when they hatched, so that alevin size at hatching was greater than expected.

Experiment 2 — The dependence of hatching time on temperature is less for Miramichi (experiment 2) than for Dennis eggs (experiment 1). The equation of the line for Miramichi data is $t = 0.74 + 0.66\ T_2$ ($r = 0.96$) and for equivalent Dennis data (those groups where $T_1 = T_2$) is $t = -4.19 + 1.20\ T_2$ ($r = 0.91$), where t is the log of the reciprocal of time from eye pigmentation to hatching. The difference in slopes is statistically significant ($P < 0.01$).

Abnormal Development

Many of the alevins reared at 2–3°C, from eggs kept at 12°C until eye pigmentation or hatching, developed severe edema of the yolk sac (Fig. 4). The symptoms closely resembled those of the so-called "blue-sac" disease. These afflicted alevins grew more slowly than did those of normal appearance and did not produce normal fry. This edematous condition is a possible reason for the higher mortality noted earlier for these groups of alevins.

Alevin Growth After Hatching

Experiment 1 — Although a number of alevins were lost from the incubation boxes in experiment 1, the data suggest, at a given posthatching temperature (T_3), that large alevins at hatching tend to maintain at least some of this length differential through completion of yolk absorption (Fig. 5). For example, in the 12°C panel of Fig. 5, the alevins held at $T_1T_2 = 4$, 4°C, then moved to 12°C (4–4 in Fig. 5) clearly were of greater length at termination of yolk absorption than those incubated at 12°C (12–12) from fertilization to hatching. Although only one measurement was taken on the latter group after hatching, little yolk remained and little further increase in length would occur. Similarly, results suggest that alevins from eggs eyed at 2°C are larger after yolk absorption than those obtained from eggs held at a constant temperature of 12°C from fertilization onward. Similar relationships are suggested in the upper and middle

TABLE 5. Experiment 1. *a*, Time from eyeing to hatching (days); *b*, mean alevin length at hatching (millimetres) (ranges in parentheses); and *c*, box number (first figure) and alevins measured (second figure) for the various combinations of eyeing (T_1) and hatching (T_2) temperatures.

		(T_2)					
(T_1)		2	4	6	8	10	12
2	*a*	77	62	37	28	22	14
	b	20.9 (20.0 –22.0)	20.3 (19.5 –21.5)	19.9 (18.5 –22.5)	18.8 (17.5 –19.5)	18.6 (18.0 –19.0)	18.0 (18.0)
	c	6, 48	1, 12	1, 10	1, 12	1, 8	1, 3
4		64	66	37	29	22	16
		20.9 (20.0 –21.5)	20.9 (19.5 –21.5)	19.3 (18.5 –20.0)	19.2 (18.5 –20.0)	18.6 (18.0 –19.5)	17.6 (16.5 –19.0)
		1, 11	6, 55	1, 12	1, 11	1, 11	1, 11
6		90	60	36.5	29	22	15
		21.5 (21.0 –22.0)	19.9 (18.0 –21.0)	19.0 (18.0 –20.0)	19.0 (18.0 –20.0)	18.9 (18.5 –19.5)	17.8 (17.5 –18.0)
		1, 10	1, 4	6, 52	1, 10	1, 8	1, 10
8		85	72	39	28	20	15
		20.5 (20.0 –21.0)	20.5 (20.0 –21.0)	19.4 (19.0 –20.0)	19.2 (17.0 –20.0)	18.5 (17.5 –19.0)	17.1 (17.0 –17.5)
		1, 10	1, 10	1, 11	6, 48	1, 11	1, 10
10		66	53	31	25	17	14
		20.3 (19.5 –21.0)	20.0 (19.5 –21.0)	18.6 (17.0 –19.0)	18.7 (18.5 –19.0)	17.9 (17.0 –19.0)	18.0
		1, 10	1, 11	1, 10	1, 10	6, 53	1, 1
12		74	51	34	20	13	9
		19.7 (18.5 –20.0)	19.5 (19.0 –20.0)	18.7 (17.5 –19.5)	18.5 (18.0 –19.0)	17.4 (17.0 –18.0)	16.6 (14.5 –18.0)
		1, 11	1, 10	1, 8	1, 3	1, 9	6, 53

TABLE 6. Lengths (millimetres) at hatching for Dennis stock (experiment 1) and Miramichi stock (experiment 2) at various constant incubation temperatures (Celsius). Figures for Dennis stock in parentheses are values calculated from the surface equation.

Temp (°C)	Dennis stock	Miramichi stock
4	20.9 (20.4)	19.3 (18.0–20.0)
6	19.0 (19.6)	18.7 (18.0–20.0)
8	19.2 (18.8)	18.4 (18.0–19.0)
10	17.9 (17.9)	17.3 (17.0–18.0)
12	16.6 (17.0)	16.2 (15.5–17.5)

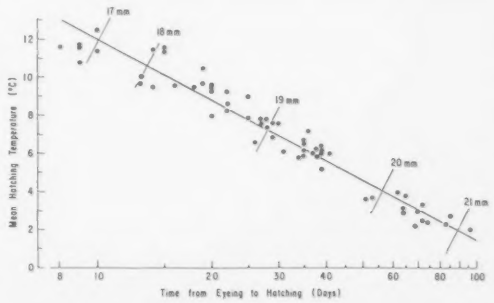

FIG. 3. The experimental points (Table 5) are combined by plotting T_2 vs. time from eye pigmentation to hatching. T_1 is neglected, but affects time to hatch and length at hatching similarly. Thus the line fitting the points is divided into segments corresponding to different hatching lengths. Equation of line — $\log t_2 = 2.18 - 0.094\ T_2$ (t_2 = time from eye pigmentation to hatching, T_2 = °C) (see text).

panels (Fig. 5) for alevins reared at $T_3 = 2$ and 6°C, respectively.

Experiment 2 — Increase in length of alevins, reared at constant temperatures from fertilization to yolk absorption, varied in several respects at the various temperatures (Table 7, Fig. 6). Length at hatching varied inversely with temperature (Table 6). As a result, in the first 10–15 days at higher temperatures, increased growth rate serves only to reduce the size difference between these and the larger alevins hatched at lower temperatures (Fig. 6). Thus, all curves tend to converge at 10–15 days after hatching. Beyond

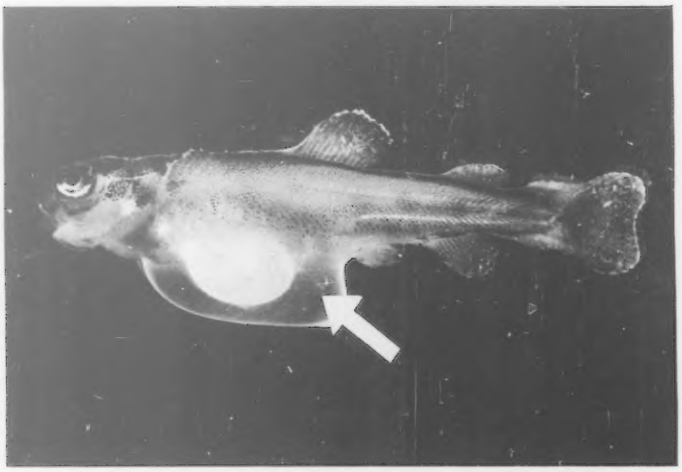

FIG. 4. Photograph of alevin afflicted with edema as a result of sudden temperature decrease at hatching. Arrow points to fluid distending vitelline membrane.

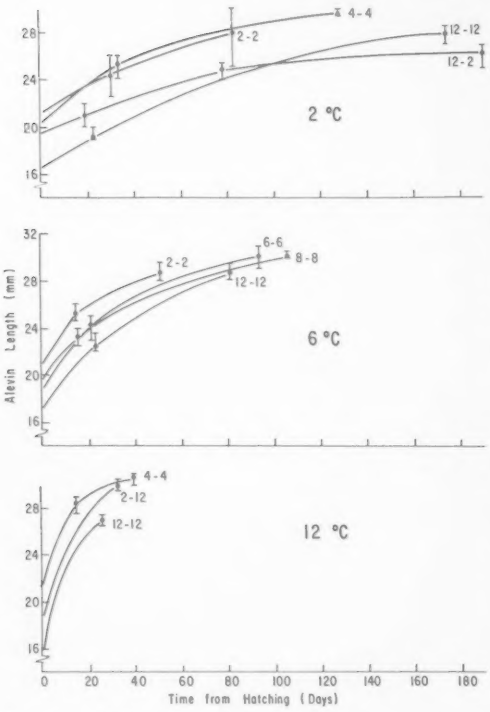

FIG. 5. Effect of previous temperature regime on increase in length at various posthatching temperatures. The two numbers associated with each line are T_1 and T_2, respectively. Temperatures for each panel are for the posthatching period (T_2).

this time, alevins at higher temperatures grow faster for another 15–20 days. About 25–30 days after hatching, the curves again begin to cross as the yolk is exhausted, and growth rate slows at the higher temperatures. The estimated maximum length attained during yolk absorption (l_∞ in Table 7) decreases above 8°C. The time required to absorb the yolk declines with increasing temperature from 88 days at 4°C to 36 days at 12°C.

Measurement of the growth rates of the alevins necessitated frequent handling and anesthesia. Two other groups of alevins were maintained at each temperature to see if the handling affected growth. In the test group at 12°C, for example, 10 alevins were measured every 5th day after hatching. In another group, measurements were made only half as often (10 days at 12°C). In a third control group no measurements were made until yolk absorption was complete. At each successive lower temperature, the time interval between measurements of the test group was increased by 1 day to allow for temperature effects on growth rate. It was concluded (Table 8) that handling did not affect growth rates in these experiments. The significant differences at 12°C are due to differences in median hatching dates, and final sizes (at 35 days) were not significantly different.

Discussion

MORTALITY

High mortality prior to eye pigmentation at temperatures below 4°C suggests that early stages

TABLE 7. Von Bertalanffy-type curves fitted to the growth patterns of Atlantic salmon (*Salmo salar*) alevins at five temperatures (Celsius). l = length (millimetres at time t, l_∞ = maximum length attained, t_∞ = time to attain maximum length. Figures for t_∞ in parentheses are interpolated values taken from a plot of log l/t_∞ vs. temperature.

Temp (°C)	Fitted polynomial	l_∞	t_∞ (*days*)
4	$l = 19.52 + 0.229t - 0.0013t^2$	29.6	88 (85)
6	$l = 18.45 + 0.368t - 0.0033t^2$	28.6	56 (62)
8	$l = 17.78 + 0.401t - 0.0034t^2$	29.6	59 (50)
10	$l = 16.88 + 0.509t - 0.0057t^2$	28.2	45 (41)
12	$l = 15.59 + 0.658t - 0.0092t^2$	27.4	36 (36)

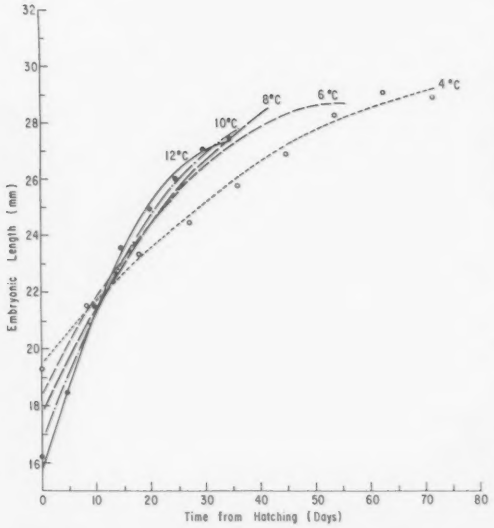

FIG. 6. Patterns of length increase with time for alevins at various temperatures. The lines are von Bertalanffy-type equations of best fit. Data are included for 4 and 12°C.

of egg development do not tolerate temperatures this low. Sensitivity to low temperatures has been reported for eggs of Pacific salmon — sockeye, *Oncorhynchus nerka* and chinook salmon, *O. tshawytscha* (Combs 1965), and pink salmon, *O. gorbuscha* (Combs and Burrows 1957). Eighty percent mortality of *O. nerka* eggs occurred at 1.7°C, with the threshold for increasing mortality occurring at about 6°C. There was no evidence of increased mortality at 4°C in the experiments described here. Combs (1965) found Pacific salmon eggs more tolerant to low temperatures beyond the 128-cell stage. None of the other temperatures used in the current experiments appear directly lethal to newly fertilized salmon eggs, although secondary fungal infections were a problem at 10 and 12°C.

The edematous conditions of alevins subjected to large temperature decreases at the eyed egg stage and at hatching should serve as a note of caution to workers rearing eggs at elevated temperatures. Failure of the heating system could be damaging, depending upon the duration and severity of the temperature decrease. The cause of the edema is unknown, but it may be associated with osmotic failure at low temperatures.

DEVELOPMENTAL TIMES AND TEMPERATURE

Development of eye pigment is not a distinct developmental stage as the embryonic stage at which it occurs can vary with temperature (Hayes 1953). It is, however, a useful operational index indicating when eggs can be moved about. Garside (1959) reported time for development of eye pigmentation in lake trout (*Salvelinus namaycust*) as 26 days at 10°C, compared with 32 and 27 days for Dennis and Miramichi salmon eggs, respectively. Rucker (1937) reported times of 30 days at 8.2°C and 21 days at 10.9°C for sockeye salmon.

A high Q_{10} is associated with hatching of Atlantic salmon eggs (Hayes 1949). This fact may be of practical benefit as it results in a wide possible choice of times when newly hatched alevins can be available. This advantage may be tempered somewhat by the fact that alevin size decreases as T_2 is raised. However, eyed eggs can be hatched at 10°C, for example, 60 days faster than at 2°C. Since complete yolk absorption after hatching requires about 40 days at 10°C, such alevins would have been feeding about 20 days by the time hatching would occur at $T_2 = 2$°C. This should more than overcome the smaller size at hatching.

TEMPERATURE AND ALEVIN SIZE AT HATCHING

Gray (1929) demonstrated that trout alevins (*Salmo fario*) were smaller at hatching at higher constant incubation temperatures. Furthermore, he demonstrated that the size advantage at low tem-

TABLE 8. Mean lengths (millimetres) of groups of 10 alevins measured at various temperatures (Celsius) and times after hatching (last column). Measurements were made at yolk exhaustion only (control) or at intervals as listed. Z values measure the significance of differences between the controls and the 10-day and 5-day measurements. For example, at 4°C, 90 days, the Z value for 5-day vs. 10-day intervals is 1.08, that for 5-day vs. control is 0. $*P < 0.05$; $**P < 0.01$.

Temp (°C)	Measurement intervals (*days*)	Length	Measurement intervals (*days*)	Length	Control	Z	Days (from hatching)
4	9	25.8	18	26.6	—	2.35*	36
4		28.2		28.2	—	0	54
4		28.8		28.7	—	0.64	72
4		28.9		28.7	28.9	1.08, 0	90
6	8	23.6	16	26.0	—	2.16*	16
6		26.8		26.3	—	1.67*	32
6		28.5		28.2	—	1.16	48
6		28.8		—	29.0	0.76	56
8	7	22.8	14	23.0	—	0.59	14
8		26.5		26.7	—	1.24	28
8		28.4		28.4	28.6	0, 0.92	42
10	6	25.6	12	25.6	—	0.10	24
10		28.0		28.3	28.0	1.21, 0	36
12	5	23.6	10	21.5	—	6.16**	15
12		26.1		24.7	—	5.70**	25
12		27.4		27.1	27.6	1.26, 0.84	35

peratures were maintained through complete yolk absorption. Similar results have been demonstrated for alevins of other families of fishes, e.g. Alderdice and Forrester (1971) for Pacific cod, Alderdice and Velsen (1971) for Pacific herring. The results described here dealing with the effects of temperature on alevin size at hatching support Gray's findings. In addition, systematic variation of temperature during egg development demonstrated that temperature from fertilization to development of eye pigmentation (T_1) had a significant but lesser effect on size at hatching than did temperature from "eyeing" to hatching (T_2). The results suggest (FIG. 3) that the effect of T_1 on hatching size is due mainly to variation in time to hatch. In other words, the reason alevins are shorter at hatching after being eyed at higher temperatures is that they hatch at an earlier stage in relation to full-term development. "Premature" hatching may also contribute to the smaller size of newly hatched alevins at the higher hatching temperatures.

The preliminary results presented here (Fig. 5) suggest, for constant posthatching temperature (T_3), that larger size at hatching (as induced by lower T_2) leads to larger size at swim-up. By varying T_3 from 12 to 2°C, the period required for alevins to reach the swim-up stage can be extended to about 100 days. It is not known whether the duration of the posthatching phase can be influenced by prehatching temperature.

Smaller size of alevins hatched at 10 and 12°C may not be solely due to direct temperature effects. Silver et al. (1963) (see Daykin 1965) perfused steelhead trout (*S. gairdneri*) eggs at 10°C, water velocities of 150 cm/h (approximately the velocities used here), and pO_2 levels approaching air saturation. The resulting alevins were approximately 0.2 mm smaller than if velocities of 740 cm/h had been used. Apparently, increased oxygen gradients at lower perfusion velocities limit oxygen uptake and reduce growth. More marked effects of water velocity on hatching size were noted for chinook salmon (Silver et al. 1963), but these eggs would be considerably larger than Atlantic salmon eggs, judging from alevin size at hatching. Hatched alevins of Atlantic salmon correspond closely in size to steelhead alevins. Temperature, per se, however, probably contributes importantly toward the smaller sizes of hatched alevins at higher temperatures for two reasons. First, the relationship between size at hatching and T_2 is linear over the entire range of temperatures tested, whereas the velocity effect should be negligible below 9°C at flow rates and pO_2 levels maintained in these experiments (from data of Silver et al. 1963). Second, the size reduction associated with the increase in T_2 from 8 to 10°C is 2–3 times the effect found for water velocity in that temperature range.

TEMPERATURE "OPTIMA"

Optimal T_1 would appear to be near 6°C, from

two points of view. First, due to the logarithmic nature of the effect of temperature on time for eye pigmentation, only 2 wk were gained by use of T_1 of 12°C as opposed to 6°C. Yet, a decrease from 6 to 4°C results in another 2-wk delay. Second, $T_1 > 6$°C resulted in smaller alevins at hatching for a given T_2, while production of eyed eggs of $T_1 < 6$°C produced alevins that were near maximum in size for a given T_2.

Selection of an "optimal" T_2 would depend upon one's definition of optimal. If one defines it in terms of rapid development to hatching, then the highest possible temperature that allows normal development is desirable. This is probably near 12°C. However at temperatures > 8°C, egg losses will probably be higher due to fungal infection. If one uses maximum alevin size at hatching (and eventually at the end of the yolk absorption) as the criterion, then the lowest T_2 consonant with normal development is desirable, probably < 2°C. At intermediate temperatures, compromises are made between rapid development and maximal alevin size. Other criteria, such as maximum biomass of marketable fish, involve factors beyond the scope of this paper.

The data presented here on development of alevins after hatching are most complete for alevins at constant temperature from fertilization to complete yolk absorption (experiment 2). Under these conditions, any advantage in incubating alevins at $T_3 = 8$°C would depend upon the point at which 12°C alevins could convert exogenous food into growth, and upon how much growth they would be capable of achieving as compared with alevins at 8°C still absorbing yolk. The advantages of higher temperatures are more apparent when examining the growth rates at $T_3 = 4$–8°C. For example, the time to achieve maximum length at 4°C is about 88 days compared with 60 at 8°C, and l_x (length at end of yolk absorption) would be only slightly less at 8°C than 4°C. The extra 30 days of feeding at 8°C would probably result in a significant gain in time for rearing fry at 8°C as opposed to 4°C.

The argument presented in the above paragraph is based upon constant incubation temperature throughout, hence smaller alevins at hatching for higher temperatures. If one were in the position of having newly hatched alevins of uniform size and were debating what temperature conditions to rear them under after hatching, then temperatures of 12°C or even higher might be desirable. The data presented here do not resolve the question of the effect of T_3 on alevin size at the end of yolk absorption. Hayes and Pelluet (1945) found growth to be more efficient at higher temperatures (42% at 0–5°C vs. 60% at 16°C).

Development Times from Fertilization and Their Relation to Thermal Units

The experimental approach taken in this work has been to separate thermal history of development of eggs and alevins into three segments: T_1 (from fertilization to development of eye pigmentation), T_2 (from eye pigmentation to hatching), and T_3 (from hatching to complete yolk absorption). Such an approach is useful in showing the effects of temperature on each segment of development and in demonstrating possible interaction among the thermal regimes of the different segments. However, total time from fertilization to hatching is also of basic interest to many researchers. Therefore, we will discuss the effects of various combinations of T_1 and T_2 on time from fertilization to hatching. Furthermore, it has been traditional in hatchery practice to use "thermal units" (i.e. cumulative temperature × time, expressed in "degree–days") to indicate developmental progress. Unfortunately, the usage is only approximate as the number of degree–days to a given stage of development is not a constant for different temperature regimes. Therefore, a discussion of the interaction between T_1, T_2, degree–days accumulated at hatching, and time from fertilization to hatching will be presented (Fig. 7).

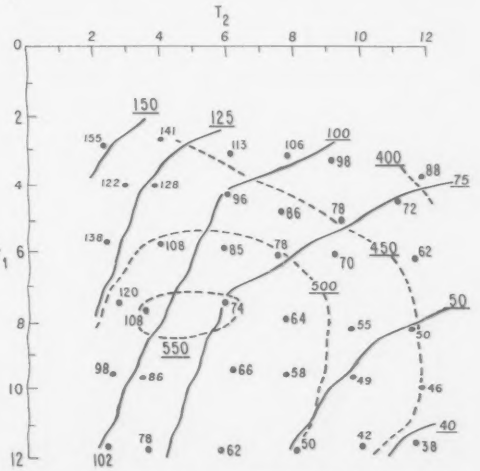

FIG. 7. Contour lines of equal time from fertilization to hatching (solid lines) and for accumulated degree–days (dashed lines) are shown for various combinations of T_1 and T_2. Smaller numbers, associated with points, are times from fertilization to hatching. Larger, underlined numbers correspond to appropriate time and degree–day isopleths.

For any isopleth of time from fertilization to hatching, there are many combinations of T_1 and T_2 possible; however, different combinations will yield different sizes of alevins at hatching. For example, alevins will hatch in about 75 days from fertilization for T_1 T_2 = 8, 6°C or 4, 12°C; however, alevins reared under the former temperature regimes will be about 2 mm longer at hatching.

Cumulative degree–days to hatching can also vary from <400 to >550 for the T_1 T_2 combinations used in experiment 1. The degree–day isopleths demonstrate a maximum occurring at T_1 T_2 = 8, 4.5°C. Several of the time isopleths (i.e. those for 50–125 days) will intersect several degree–day lines. Along any given time isopleth, alevin size at hatching appears positively correlated with the number of degree–days accumulated. For example, following the 100-day isopleth from lower left to upper right, initial alevin length is about 20.0 mm, and the degree–days accumulated are slightly over 500. Alevin length increases by about 0.5 mm and degree–days rise to a maximum of over 550 at T_1 T_2 = 8, 4°C. Accumulated degree–days at hatching and alevin length then decrease as we move along the isopleth to about 425 degree–days and <19 mm at T_1 T_2 = 3, 9°C. There is, therefore, an interaction between time from fertilization to hatching, degree–days accumulated, alevin lengths at hatching, and temperature. For longer times from fertilization to hatching, fewer degree–days are required to produce alevins of a given size. At 100 days from fertilization to hatching, <450 degree–days are required to produce alevins averaging 18.6 mm long at hatching. At 50 days, on the other hand, over 500 degree–days are required to produce alevins of similar length at hatching.

Natural Temperature Regimes

Results obtained in these experiments can be compared with those expected from natural temperature regimes to which salmon eggs and alevins are exposed in redds. Spawning normally occurs on the Northwest Miramichi in mid- to late October when stream temperatures are about 6°C and falling. Although temperature records (Curventon fence records extend from mid-April until mid-November) are scanty through the winter (probably near 0.1°C), estimation of time of eye pigmentation is in mid- to late April when temperatures are between 1 and 2°C. Temperatures begin to rise rapidly in late April, and hatching probably occurs typically around 6°C during the 2nd or 3rd wk in May. Temperatures continue to rise rapidly in the period after hatching and may exceed 10°C at emergence from the gravel in May. Assuming adequate aeration of the eggs and alevins, size of hatched alevins from this natural temperature regime may be compared with the data obtained from these experiments. The best approximation of natural temperatures is a T_1 of 0.5–1°C and a T_2 ca. 3–4°C. Under these conditions for equivalent-sized eggs, the hatched alevins would be about 21 mm in size. If the trends shown in Fig. 5 are real, then the large size at hatching would result in emergent fry of near maximum size.

Miramichi vs. Dennis Stocks

Differences were obtained between eggs of Dennis and Miramichi stocks with respect to time to eye pigmentation at various temperatures and to temperature dependence of development from eye pigmentation to hatching. Miramichi eggs developed eye pigmentation more rapidly at all experimental temperatures, and they had a lower temperature dependence for rate of development from "eyeing" to hatching than did Dennis stock. Caution must be used in interpreting these findings, particularly since the Dennis eggs were the progeny of a single pair, and experiments with the two lots of eggs were performed in different years. Such differences, however, are what one would expect if the differences obtained resulted from adaptation of Miramichi stock to a cooler environment (Prosser 1965). These differences could conceivably be due to differences in temperature dependence of development, or different Q_{10}'s of the hatching enzyme.

In conclusion, if one were to select a single "best" incubation regime for Atlantic salmon eggs (for aquaculture purposes), then T_1 = T_2 = 6–7°C would be the choice. This regime would produce maximum numbers of alevins which would hatch in a "reasonably" short period of time (ca. 75 days). It maximizes the degree–days, and therefore alevin size, for this incubation time (Fig. 7). Also, the advantage of large alevin size at incubation temperatures <6°C may be offset by the earlier exogenous feeding and faster growth of alevins raised at higher temperatures (8–12°C) after hatching.

Acknowledgments

We wish to thank Mr L. R. MacFarlane, Dr P. F. Elson and his field crew for assistance in obtaining eggs of Dennis origin. The Department of Environment fish hatchery at South Esk, N.B. under the management of Mr A. W. Winn provided the eggs of Miramichi origin. Mr P. W. G. McMullon and Mr F. Cunningham prepared the figures. Drs A. M. Sutterlin and D. F. Alderdice reviewed the manuscript.

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Age Determination of an Elasmobranch (*Squalus acanthias*) by X-ray Spectrometry

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JONES, B. C., AND G. H. GEEN. 1977. Age determination of an elasmobranch (*Squalus acanthias*) by X-ray spectrometry. J. Fish. Res. Board Can. 34: 44–48.

Ages of spiny dogfish (*Squalus acanthias*) in the Strait of Georgia, B.C., have been estimated by an X-ray spectrometric technique which involves measuring variations in the element composition within vertebrae.

JONES, B. C., AND G. H. GEEN. 1977. Age determination of an elasmobranch (*Squalus acanthias*) by X-ray spectrometry. J. Fish. Res. Board Can. 34: 44–48.

Une méthode de spectrométrie aux rayons X, comportant la mesure des variations dans la composition élémentaire des vertèbres, nous a permis d'estimer l'âge d'aiguillats communs (*Squalus acanthias*) du détroit de Georgie, C.-B., Canada.

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THE age of many fishes can be determined by examining scales, otoliths, or bones for evidence of seasonal changes in deposition of hard tissue. Since most of these means are not applicable to elasmobranchs, age determinations have been attempted in other ways. The only method available, other than tagging or length-frequency analysis, which are time consuming, expensive, or inaccurate techniques, has been visual examination of patterns of deposition in hard tissue. In this group spines are uncommon, teeth are constantly renewed, scales are diminutive, and most of the skeleton is cartilage. Calcified vertebrae are the only hard tissue components consistently present. Ishiyama (1951), Daiber (1960) and Berry (1965) noted circuli in vertebral cross sections of skates, as did Haskell (1949), Aasen (1963), and Stevens (1975) for several species of sharks.

In this study we attempted to determine ages for the spiny dogfish shark (*Squalus acanthias*) in which vertebral circuli have not been observed (Kaganovskaia 1933, 1937). Since growth of the elasmobranch skeleton is a one-way process of deposition (Simkiss 1974) in which no internal remodeling or resorption of minerals apparently occurs, seasonal or annual variation in deposition might still be expected. This could result from a change in metabolic uptake of different elements as environmental conditions change seasonally, either as a function of biological activity or element availability. Thus, food consumption or availability might limit the uptake of a particular element, or environmental factors may differentially affect uptake of elements from water. Though such quantitative differences in deposition of elements in dogfish vertebrae are not visible, they might be detectable by step-wise microanalysis. In this context X-ray spectrometric measurement may offer a new method of age determination.

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Materials and Methods

The spiny dogfish used in this study were collected in 1974 and 1975 from the Strait of Georgia off Namaimo, B.C. The largest vertebrae were selected, those immediately anterior to the first dorsal fin. Excess tissue was removed and vertebrae were separated and frozen. Though we used only frozen material, 24-h fixation in alcohol had no demonstrable effect on element composition. However, preservation in Formalin or Bouin's solution eroded the embedded area and reduced the measured age. Subsequent preparation involved freeze-drying the vertebrae for 24 h and shaving one end with a razor blade to remove the intervertebral cartilage and expose the embedded area (Fig. 1B). A stained sagittal section through this region (Fig. 1A and C) indicated that this procedure transected all the tissue layers. Vertebrae were then glued to aluminum sample stubs and coated with a microlayer (approximately 50Å) of carbon to minimize the possible effects of surface charging which could deflect the electron beam or reduce repeatability of measurements. The sample and glue showed no evidence of contamination.

The equipment used in element analysis consisted of an ETEC Autoscan scanning electron microscope, a Nuclear Semi-Conductor lithium-drifted silicon detector, and a Northern Scientific Econ II pulse height analyzer. This is an energy-dispersive X-ray spectrometric system in which electrons striking the sample produce X-rays, some of which pass through

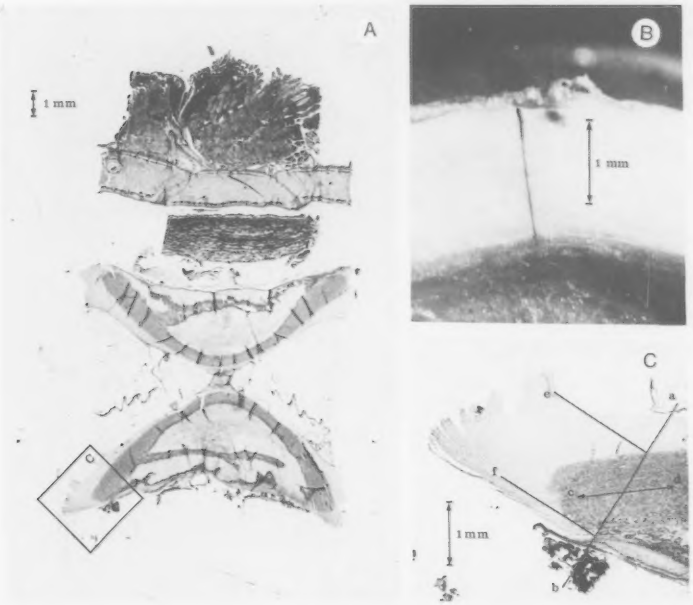

FIG. 1. Dogfish vertebrae used for X-ray spectrometry in A, sagittal section indicating embedded area (stained); B, cross section showing scan range (dark line caused by electron beam); and C, tissue orientation. ab = cut for sample preparation of whole vertebra, cd = direction of tissue fibers in embedded area, and ef = scan range.

a collimator to the detector (Fig. 2). The current pulse thus generated is relayed through amplifiers to the analyzer which has a series of memory units (channels) that divide the energy spectrum under consideration into equal intervals. The energy level of the X-rays produced from each element differs; higher emission energies for the same spectral lines result from elements with higher atomic weights. The more abundant elements in the sample are represented by peaks above a background radiation level in a particular channel range on the cathode ray tube display of the analyzer. The integrated area under each peak is determined by summation of individual channel data and can be read from an additional visual display. Further details on the theory and operation of this type of system are presented by Woldseth (1973).

In this work a collimator with a 1 mm diam aperture was fixed at approximately 70° off the vertical electron beam (Fig. 2). A larger aperture decreased sensitivity by allowing the passage of more background X rays, and a smaller one decreased the count rate, thereby necessitating increased analysis time. The sample position was adjusted on its three-dimensional axis such that the excitation area was in line with the collimator. For excitation purposes an accelerating voltage of 30 kV with a sample current of approximately 1.0×10^{-9} A yielded optimum X-ray production. Maximum count rate for this current was obtained when the sample was inclined towards the collimator at 45° off vertical producing a take-off angle of about 25° from the sample axis. At the magnification used in this study excitation areas ranged from 20 to 100 μm^2.

The energy spectrum within which elements could be readily analyzed with this system encompassed approximately 40 elements bounded by sodium at the lower level and tin (near beam energy) at the higher level. These bounds relate to the efficiency of our detection system. Silicon and aluminum could not be used reliably as they form part of the detector construction materials. The ability of specific types of equipment to reliably analyze different elements is further discussed by Rhodes (1966).

The sample was rotated to align the desired part of the scan range (embedded area) with the horizontal stage axis most nearly perpendicular to the sample-collimator direction (Fig. 2). This was necessary as the sample tilt was on the latter axis and moving it resulted in the electron beam striking the sample at a different vertical position. Preliminary analysis resulted in distinct peaks with an analysis time of 100 s/area. Integration gates were set around each peak at background level so that the corrected number of counts under their curves (peak areas) could be determined by subtracting the mean background from the total between the gates.

Analysis was begun with low magnification at the

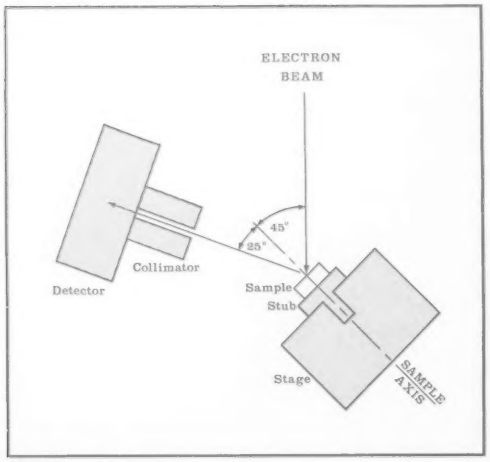

FIG. 2. Geometric arrangement of our sample and electron microscope chamber components for optimal X-ray production. Stage axis used in analyses was perpendicular to plane of figure.

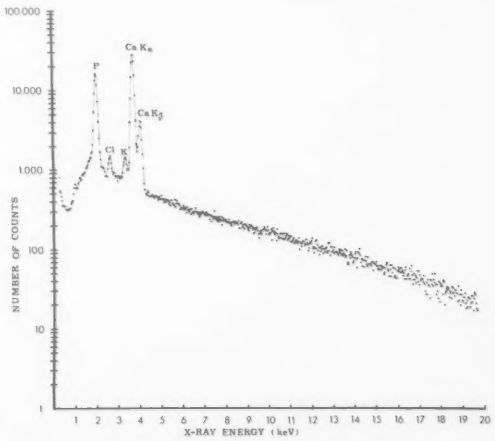

FIG. 3. Typical data plot for a 100-s analysis within the embedded area of a dogfish vertebra. P = phosphorus, Cl = chlorine, K = potassium, and Ca = calcium (K_α and K_β are different X-ray emmission energies for the same element resulting from replacement of electrons dislodged by the microscope beam by ones from different orbitals).

outer edge of the embedded area (scan range), where any changes in element deposition might be closer together reflecting slower growth with increasing age. The range was traversed (Fig. 1B) with nonoverlapping sequential areas by micrometer movement of the stage. The micrometer increment was then reduced and the magnification increased to allow more analysis areas within the scan range. This process was repeated until cyclic variations in elements were detected and were only verified by further increases in magnification.

Following the scan, the corrected peak areas were plotted and the number of cycles counted and used as an index of age. The statistical validity of observed cycles was established by reference to the possible range of equipment counting error. Using a brass standard, count variability at the level used in this study produced a 95% confidence range of ±0.9% of the corrected count level for copper and zinc over 500 s. As error increases with decreased counts, an additional method of error computation was employed. In this formula 95% confidence limits were $A \pm 1.96\sqrt{TA}$, where A was the corrected count for the element and TA was the uncorrected count (Woldseth 1973). In any single analysis, the largest of these error calculations was used in evaluating cycle peak–trough differences. We assumed that differences had to exceed twice the error at the 95% confidence limit level to be considered valid.

Results and Discussion

In dogfish vertebrae, calcium and phosphorus were the only elements clearly separated by the X-ray spectrometric method (Fig. 3). A $CaHPO_4$ standard verified that calcium and phosphorus peaks were correctly identified. At the ends of each scan the peak areas of both elements were reduced to background level as the electron beam struck the surrounding cartilage. To determine sample repeatability, the patterns of elemental composition within the vertebrae were analyzed twice, with the second scan lying roughly horizontally at right angles to the first. In each case, the number of cycles determined was identical in any one vertebra.

The cyclic pattern of these elements observed in dogfish vertebrae (Fig. 4) suggested seasonal changes in rates of deposition. Some confirmation of this postulate was obtained by comparison of ages of young dogfish estimated from X-ray spectrometry with those derived from length-frequency data. Length-frequency data indicated that 1- and 3-yr-old individuals exhibited single and triple cycles of calcium and phosphorus in their vertebrae, thus indicating a yearly period in these changes. To further examine this observation, the temporal and spatial variation of the chosen elements in the environment and the possible effect of climatic fluctuations on uptake were considered. In British Columbia coastal waters phosphorus concentrations characteristically exhibit winter maxima and summer minima, while calcium, which is relatively abundant, remains practically constant. Hence, phosphorus availability appears to have a yearly period. Since the X-ray spectrometric patterns of calcium and

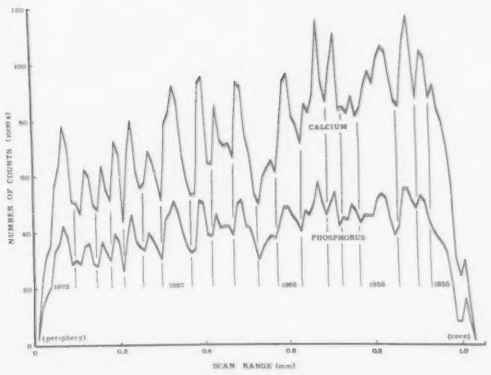

FIG. 4. Element count pattern for CaK_α and P in the vertebra of an 83-cm Strait of Georgia male dogfish. Confidence limits of 95% as derived by text method exist about each curve but are evident only on peaks and troughs. Dates are derived by equating the peripheral peak as the last complete year of growth and working backward through subsequent peaks. Since the young are normally released in November, and this animal was caught in June, 1974, an age of 20.6 yr is suggested.

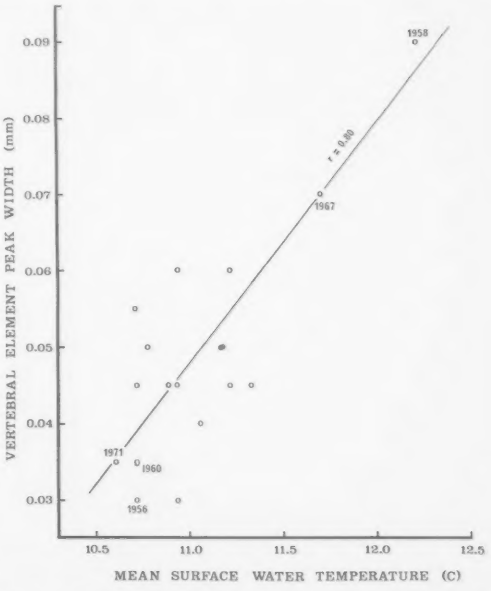

FIG. 5. Relationship between vertebral element peak width (distance between adjacent minima) as determined by X-ray spectrometry and mean annual surface water temperature at Departure Bay, B.C., for the dogfish noted in Fig. 4.

phosphorus are similar (Fig. 4), possibly because phophorus may have some direct bearing on the uptake of calcium (Love 1970), the regular changes in calcium also appear to be annual, suggesting that age may be estimated by counting the cycles.

The widths of cycle peaks (expressed as distance between adjacent minima) also varied. Since dogfish are poikilotherms these changes could reflect environmental conditions, such as seasonal temperature changes, during the main periods of growth. To examine this hypothesis, dates were assigned to each peak by equating the peripheral one as the last complete year of growth and assigning years to each previous peak. Though the normal depths inhabited by dogfish are poorly known, when peak widths are compared (Fig. 5) with mean annual surface water temperature at Departure Bay, B.C., the area with records closest to that from which the dogfish were taken, a significant correlation was found ($r = 0.80$, $P = 0.001$, $N = 18$). In warm years, such as 1958 and 1967, the peak widths were distinctly wider than in cooler years (1956, 1960, and 1971). These data further suggest that the cycles of calcium and phosphorus are annual.

The spiny dogfish is also among the few living elasmobranchs to have dorsal fin spines. These have been employed in an alternative technique in which the circuli have been counted as a measure of age (Ketchen 1975). The age–length relationship obtained for Strait of Georgia dogfish by this method is similar to that determined using X-ray spectrometry (B. C. Jones and G. H. Geen unpublished data), again supporting annual interpretation for the observed cycles of calcium and phosphorus.

Regarding the major problems associated with X-ray spectrometric analysis, tests did not indicate any appreciable effects of sample topography as indicated by insignificant differences among analyses of a specific area from several opposite aspects. Similarly, intermittent analysis of a particular area over long periods of time showed no significant alteration of composition or vacuum system contamination. Interelemental absorption or enhancement as a possible source of error was not taken into consideration for economic reasons. The corrected peak areas should also be normalized in relation to variations in beam current at the specimen level using the following formula:

$$A_n = A/[(S_c1 + S_c2)/2]$$

where A is the corrected count (peak area) for the element, A_n is the normalized area, and S_c is the specimen current at the beginning and end of

each analysis. In our study, this was not done as it would have involved extensive effort, and preliminary examination indicated sample current drift at the sample level to be generally slow (0.01×10^{-9} A/min), and thus would likely have little effect on area counts over the short time intervals required to delineate adjacent peaks and troughs. The general increase in cycle height from periphery to core as seen in Fig. 4 could have resulted from current drift, but this was not verified. Though these possible sources of error may negate a precise statement regarding changes in concentrations of the elements examined in dogfish vertebrae, the variations reflected in peak area changes are nonetheless statistically significant.

This study indicates that the X-ray spectrometry technique may provide a measure of elasmobranch age which can be used to estimate growth rates. However, the extent of its use obviously relates to the time and money available, since it is a time-consuming procedure. For example, at the 100-s analysis rate it takes approximately 1 h to scan the vertebra of a 1-yr-old animal, and 6 h for a 30-yr-old. However, it is possible that the analysis rate could be improved by using a continuous sample-stage movement in conjunction with an automatic analyzer relay to a computer-plotter. This would reduce the time required and minimize human error. A system possibly adaptable to this use has been outlined by Dao (1974). Though age determination by X-ray spectrometry has been applied to spiny dogfish in this study, it might be applicable to other fish species where no alternative is more suitable. Theoretically, it might be used for determining the age of any organism possessing a hard tissue subjected to environmental changes during its growth, provided the periodicity is known.

Acknowledgments

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A Utility Function for Examining Policy Affecting Salmon on the Skeena River

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KEENEY, R. L. 1977. A utility function for examining policy affecting salmon on the Skeena River. J. Fish. Res. Board Can. 34: 49–63.

The interests of many groups, some with multiple objectives, are important to include in evaluating strategies affecting salmon in the Skeena River. A multiattribute utility model is proposed for addressing these issues. Two first-cut utility functions are assessed using the preferences of two individuals familiar with the problem. These utility functions provide a basis for constructive discussion to arrive at a reasonable utility function for examining alternative policies. Two unique features of this study are the explicit focus on value tradeoffs and equity considerations among interest groups, and a comparative examination of the two first-cut multiattribute utility models. This examination indicates the range of fundamental preferences which can be captured using multiattribute utility functions and illustrates the potential of the theory for conflict illumination and resolution.

KEENEY, R. L. 1977. A utility function for examining policy affecting salmon on the Skeena River. J. Fish. Res. Board Can. 34: 49–63.

Dans l'évaluation de stratégies affectant le saumon de la rivière Skeena, il est important de tenir compte des intérêts de plusieurs groupes, dont certains ont de multiples objectifs. Nous proposons un modèle d'utilité à multiples attributs pour traiter de ces questions. Nous évaluons deux fonctions d'utilité de première taille en nous basant sur les préférences de deux individus familiers avec le problème. Ces fonctions fournissent une base à une discussion constructive qui conduira a ùne fonction d'utilité raisonnable dans l'examen de politiques alternatives. Deux caractéristiques uniques de cette étude sont d'abord l'importance donnée aux échanges de valeurs et aux considérations de justice parmi les groupes d'intérêt et ensuite un examen comparatif des deux modèles d'utilité à multiples attributs de première taille. Cet examen donne une indication de la gamme de préférences fondamentales qui peut être captée en utilisant des fonctions d'utilité à multiples attributs et démontre le potentiel de la théorie à faire ressortir et à résoudre des conflits.

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1. Introduction

THE Skeena River, and its tributaries in British Columbia, Canada, is an important salmon fishing area. Salmon fishing, which is the basis of the area's economy, currently provides around 5,000 jobs. This includes the fishermen themselves, people working in canneries, and individuals earning a living from tourism as a result of the recreational fishermen. Policy decisions indicating, for example, 1) who can fish, 2) what they can fish (types or size of salmon), 3) where they can fish, 4) which methods they can use, 5) when they can fish, impact directly or indirectly everyone living in the Skeena area. Other options such as the development of artificial spawning grounds are also possible and need to be evaluated. Such possibilities have many parameters (size, construction type, cost). Even if one decides to construct spawning grounds, how should they be designed?

The decision maker for such policy problems is the Canadian Department of the Environment (DOE). The problems are both important and complex. Three crucial aspects of the complexity are:

1) Any decision (or no decision) impacts several groups, and these groups have interests directly in conflict with each other.

2) Some of these groups themselves have multiple conflicting objectives.

3) The uncertainties about the consequences of any decision are large.

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This article illustrates an approach — multiattribute utility analysis — which allows one to formally address these complexities. Multiattribute utility examines the preference and value tradeoff aspects of the problem. For instance, it will indicate "how much" DOE should be willing to take away from group A to give a specified amount to group B. To be used directly in evaluating options, one also needs a model to indicate the impacts of the various options on groups A and B. A simulation model has been developed by the Ecology Project at the International Institute for Applied Systems Analysis (IIASA) to do the latter (see Walters 1975).

We will concentrate only on the preference model, called a multiattribute utility function or, more simply, just a utility function. A utility function is nothing more than an ordinary objective function (to be maximized) except for one special property: in selecting among alternatives involving uncertainty, the expected utility is an appropriate indicator of the desirability of the alternative. If one accepts a set of reasonable assumptions first postulated by von Neumann and Morgenstern (1947), then the decision maker should choose the alternative with the highest expected utility. Raiffa (1968) discusses the implications of these assumptions and argues for their reasonableness for selecting among alternatives.

The paper is organized as follows. Section 2 structures the preference aspects of the problem. The interests of the various groups are indicated. Section 3 briefly introduces aspects of multiattribute utility theory used in this study. Sections 4 and 5 form one unit. The fourth section indicates one possible first-cut utility function for each of the interest groups. In Section 5, the preferences of these groups are used as inputs to construct a utility function for the DOE. Sections 6 and 7 also form a unit and construct an alternative utility function for the DOE. In Section 8 we discuss the differences in basic preference attitudes implied by the two utility functions. The final section suggests ways in which a multiattribute utility analysis may help in examining options for the Skeena River.

One should interpret the results in this paper as preliminary. The utility functions were assessed quickly and roughly using rather standard methodology (see Section 3) to provide a basis on which to improve and to provide the component utility functions necessary for constructing a first cut of DOE's preferences. All the assessments are based on discussions with Dr R. Hilborn (Sections 4 and 5) and Dr C. Walters (Sections 6 and 7) formerly of the IIASA Ecology Project. Both Hilborn and Walters are from the University of British Columbia and are working on a model of salmon in the Skeena River. In making the utility assessments, each used his knowledge of the "Skeena Problem" to respond in the way he expected the groups and DOE to respond.

There are two unique aspects of the study. The first involved synthesizing the utility functions of the separate groups into an overall DOE utility function. This required the explicit consideration of value tradeoffs between groups and of equity among the groups. The second unique feature involved the comparative examination of Dr Hilborn's and Dr Walters' utility assessments. This clearly identifies points of agreement and disagreement concerning the preference structure (i.e. objectives) to be used in the study. Similar analyses, done in more detail with more care, could assist in identifying and resolving conflicts in problems with multiple decision makers. Of course, before any complete evaluation of DOE policy with such a procedure, attempts should be made to get the actual groups' utility functions. This is intended in the course of the overall Skeena study (see Hilborn and Walters 1977).

2. The Interest Groups and Their Objectives

There are five main groups whose preferences are important to DOE. Four of these — the lure fishermen, the net fishermen, the sport fishermen, and the Indians — are directly involved in fishing. The fifth group includes all those individuals whose welfare is tied to fishing, such as the cannery employees and motel operators. Let us refer to just the latter group as the "regional development" group.

The lure and net fishermen fish for a livelihood, using lures and nets, respectively. The lure fishermen are near the mouth of the Skeena, and the net fishermen are upstream a little in a controlled area. Upstream from them are the sport fishermen and upstream still are the Indians. The latter two groups fish mainly for pleasure and food.

The objectives hierarchy for this problem is illustrated in Fig. 1. DOE, as decision maker, has two major objectives: to satisfy each of the five interest groups as much as possible and to minimize their own (government) expenses. The degree to which the net fishermen are satisfied depends, of course, on how well their own objectives are satisfied. As indicated in the figure, their main interests are to maximize their income per net fishermen, optimize their fishing time (i.e. don't work too much or too little), and maximize the diversity of the catch. The last objective is a proxy indicator for their psychological well-being.

TABLE 1. Attributes for the Skeena problem.

Attribute	Worst level	Best level
X_1 = annual income/net fisherman	0	$25,000
X_2 = annual days fishing/net fisherman	100	0
X_3 = species of salmon in the Skeena	1	10
X_4 = annual income/lure fisherman	0	$25,000
X_5 = annual days fishing/lure fisherman	100	0
X_6 = species of salmon in the Skeena	1	10
X_7 = annual sport fisherman catch of salmon	0	1,000,000
X_8 = employment	0	5,000
X_9 = annual revenue due to recreation	0	$10,000,000
X_{10} = cost of fresh salmon/lb	$10.00	$0.20
X_{11} = annual Indian catch of salmon	0	100,000
X_{12} = annual expenditures (millions of dollars)	$10	$0

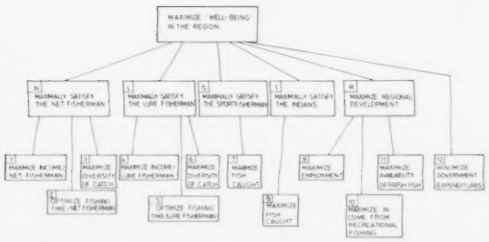

FIG. 1. The objectives in the Skeena problem.

Knowing the river is healthy (i.e. supporting many species) provides both future flexibility and future security. The lure fishermen have analogous objectives. The Indians and sport fishermen are interested in maximizing their fish catch. The region wants to maximize economic benefits from employment and recreational sources as well as to have an abundance of fresh fish to eat.

Let us return to the three complexities mentioned in the introduction and elaborate on them in the context of our problem. Suppose DOE is considering implementing an innovative licensing policy. One scenario that may result from that policy is that only a few additional fish will return from the ocean, whereas another scenario may lead to large increases in adult salmon in the Skeena. The uncertainties here are large. If DOE changes licensing strategies, this may increase administrative (government) costs and simultaneously lead to better harvests for lure and net fishermen. However, there may be less fish for the sport fishermen and the Indians. These groups would then be displeased. The overall impact on the region might be more employment in canneries, etc., but less recreational income. What should DOE do? Somehow they must get a measure of each of the possible impacts, balance these in some fair way, and decide, "with all pros and cons considered, should we go ahead with the new licensing strategy or not?"

3. Methodology

For each of the objectives on the bottom level of the DOE's hierarchy, Table 1 lists a measure, called an attribute, as well as ranges of the possible impacts on each attribute. We will designate $X_i, i = 1,2 \ldots, 12$ as the attribute associated with the i^{th} objective, and use x_i to indicate a specific level of attribute X_i. Then the consequence of any decision taken by DOE can be represented by the twelve-tuple $(x_1, x_2, \ldots, x_{12})$. For decision making, we want to assign a number $u_G(x_1, x_2, \ldots, x_{12})$, referred to as utility, to each consequence. One may think of u_G as DOE's utility function. Rather than assess u_G directly, we will break the problem into parts using the structure

$$u_G(x_1, x_2, \ldots, x_{12}) = u[u_N(x_1, x_2, x_3), u_L(x_4, x_5, x_6), u_S(x_7), u_R(x_8, x_9, x_{10}), u_I(x_{11}), x_{12}] \quad (1)$$

where u_N, u_L, u_S, u_R, and u_I are utility functions for the net fishermen, lure fishermen, sport fishermen, region, and Indians, respectively, x_{12} is DOE's expenditures, and u is itself a utility function over the six attributes U_N, U_L, U_S, U_R, U_I, and X_{12} which take on amounts designated by $u_N, u_L, \ldots, x_{12}$.

The three multiattribute utility functions u_N, u_L, and u_R are also divided into their component parts, so

$$u_N(x_1, x_2, x_3) = f[u_1(x_1), u_2(x_2), u_3(x_3)] \quad (2)$$

$$u_L(x_4, x_5, x_6) = g[u_4(x_4), u_5(x_5), u_6(x_6)] \quad (3)$$

and

$$u_R(x_8, x_9, x_{10}) = h[u_8(x_8), u_9(x_9), u_{10}(x_{10})] \quad (4)$$

where each u_i is a utility function over the attribute X_i.

To structure the utility functions as indicated in (1)–(4), one needs to make some assumptions. The two assumptions used in this study are preferential independence and utility independence. Let us briefly define these and state two results following from them which are used in structuring DOE's preferences.

Assume we have the set of attributes $\{Y_1,Y_2, \ldots ,Y_n\}$. If $n \geq 3$, we will say the pair of attributes $\{Y_1,Y_2\}$ is preferentially independent (PI) of the other attributes if the preference ordering of (Y_1,Y_2) pairs, given the other attributes are held fixed, does not depend on the levels where they are fixed. If $\{Y_1,Y_2\}$ is PI, then the value tradeoffs between Y_1 and Y_2 won't depend on the levels of Y_3 through Y_n.

Whereas preferential independence concerns only preferences for sure consequences, utility independence concerns preferences for lotteries. A lottery indicates which of several consequences may occur and an associated probability of the occurrence of each. We will say Y_1 is utility independent (UI) of the other attributes if the preference order for lotteries over Y_1, given the other attributes are fixed, does not depend on the level where those attributes are fixed.

Given various preferential and utility independence conditions, one can derive utility functions consistent with the decompositions (1)–(4). For purposes here, we are interested in two particular results:

Theorem 1. Given $Y_1,Y_2, \ldots ,Y_n$, $n \geq 3$, suppose for some Y_i, both $\{Y_i,Y_j\}$ is PI for all $j \neq i$, and Y_i is UI, then either

$$u(y_1, y_2, \ldots, y_n) = \sum_{i=1}^{n} k_i u_i(y_i), \text{ if } \sum k_i = 1 \quad (5)$$

or

$$1 + ku(y_1, y_2, \ldots, y_n) = \prod_{i=1}^{n} (1 + kk_i u_i(y_i)), \quad \text{if } \sum k_i \neq 1 \quad (6)$$

where

i) u and the u_i are utility functions scaled 0 to 1, (7)

ii) $0 < k_i < 1, \quad i = 1, 2, \ldots, n$ (8)

and if $\Sigma k_i \neq 1$, $k > -1$ is the nonzero solution to

iii) $$1 + k = \prod_{i=1}^{n} (1 + kk_i) \quad (9)$$

Theorem 2. Given Y_1 and Y_2, if Y_1 is UI and Y_2 is UI, then either (5) or (6) holds. With our scaling convention, both cases can be written as

$$u(y_1, y_2) = k_1u_1(y_1) + k_2u_2(y_2) + (1 - k_1 - k_2)u_1(y_1)u_2(y_2) \quad (10)$$

Proof of these results as well as more details about preferential independence, utility independence, and related results are found in Keeney (1974) and Keeney and Raiffa (1976).

4. Hilborn's First-Cut Utility Functions for the Interest Groups

In this section we illustrate the assessment of Hilborn's utility functions of the five interest groups. Our first assessment session consisted of examining the reasonableness of the preferential independence conditions required to use (2), (3), and (4). At that time a fourth attribute called freedom, measured by the number of boats fishing an area, was included in both the net fishermen's and lure fishermen's preferences. It turned out that the fishermen would give up more in terms of annual salary to avoid large congestion (many boats) if annual days of fishing were high than if they were low. Thus, freedom combined with income, for example, was not preferentially independent of days of fishing. However, preliminary analysis indicated that the weight of the freedom attribute, given its possible ranges, would likely have little impact on evaluating policy. Hence, it was dropped. This left us with the three attributes in Fig. 1 for the two commercial fishermen's groups.

The Net Fishermen's Utility Function

For attributes X_1, X_2, and X_3 the preferential independence conditions necessary to use Theorem 1 seemed reasonable. Thus, we know that either

$$u_N(x_1, x_2, x_3) = k_1u_1(x_1) + k_2u_2(x_2) + k_3u_3(x_3) \quad (11)$$

or

$$1 + k_N u_N(x_1, x_2, x_3) = (1 + k_N k_1 u_1(x_1)) \times (1 + k_N k_2 u_2(x_2))(1 + k_N k_3 u_3(x_3)) \quad (12)$$

where u_N, u_1, u_2, and u_3 are scaled from 0 to 1 and k_i is defined as the utility measured by u_N of attribute X_i at its best level and the other two attributes at their worst levels for both (11) and (12). If $k_1 + k_2 + k_3 = 1$, then (11) holds. Otherwise, the number k_N is calculated directly from k_1, k_2, and k_3. Thus to specify $u_N(x_1,x_2,x_3)$, we needed to assess u_1, u_2, u_3 and k_1, k_2, and k_3.

The assessment of u_i, $i = 1,2,3$ followed procedures discussed in Keeney and Raiffa (1976). To illustrate briefly concerning X_1, we found that

the average of any lottery is preferred to the lottery itself. This implied u_1 was concave. Then the certainty equivalent[2] for the lottery <\$0; \$25,000> yielding a 50–50 chance at an annual salary of \$0 or \$25,000 was found to be \$8,000. Thus, $u_1(8{,}000)$ must equal the expected utility of the lottery so

$$u_1(8{,}000) = .5u_1(25{,}000) + .5u_1(0) = 0.5$$

which together with our scaling convention gives us the three points on the graph in Fig. 2A. An exponential utility function was fit through these three points. As a check, we assessed \$3000 indifferent to <\$0;\$8,000> which seemed quite consistent.

Similar exponential fits to the three points indicated in Fig. 2B and C were used for attributes X_2 and X_3. Part of the justification for the use of this simple form is that more sophisticated forms add little additional flexibility in a multiattribute context relative to their additional complexity.

Next we wanted to assess k_1, k_2, and k_3 in as simple a way as possible. This was done with the information in Fig. 2D–F. First, we considered tradeoffs between X_1 and X_2 given X_3 was held fixed: ($x_1 = 0$, $x_2 = 0$) and ($x_1 = \$7000$, $x_2 = 100$) indicated in Fig. 2D were indifferent. Equating the utility of these two consequences using either (11) or (12) yields

$$k_2 = k_1 u_1(\$7{,}000) \tag{13}$$

Similarly, looking at tradeoffs between X_1 and X_3, we found ($x_1 = \$3{,}000$, $x_3 = 1$) and ($x_1 = 0$, $x_3 = 10$) indifferent. Thus

$$k_3 = k_1 u_1(\$3{,}000) \tag{14}$$

Finally, we assessed the probability p_N such that the consequence and the lottery in Fig. 2F were indifferent. This was found to be $p_N = 0.8$. Equating utilities of the consequence and the lottery yields

$$k_1 = 0.8 \tag{15}$$

Solving the three equations (13), (14), and (15) using u_1 from Fig. 2A, we find $k_1 = 0.8$, $k_2 = 0.36$, and $k_3 = 0.17$. Since $k_1 + k_2 + k_3 \neq 0$, the multiplicative utility function (12) is the appropriate one.

We ran two consistency checks on the assessments. The first involved choosing which of the two lotteries in Fig. 2G was preferable. In each case there is a 50–50 chance of getting 0 or \$25,000 for the year and a 50–50 chance of working 0 or 100 days. The difference is in how the two attributes are combined. The second lottery was preferred, which implies $k_1 + k_2 + k_3$ must be greater than one. This check was consistent.

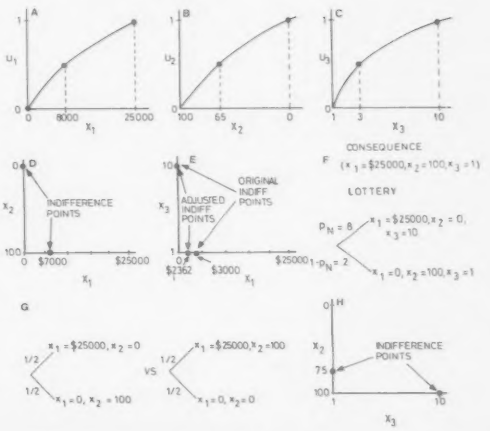

FIG. 2. Assessing Hilborn's utility function for net fishermen.

The second check involved considering tradeoffs between X_2 and X_3. We found ($x_2 = 75$, $x_3 = 1$) and ($x_2 = 100$, $x_3 = 10$) to be indifferent as indicated in Fig. 2H. Equating utilities implies

$$k_3 = k_2 u_2(75) \tag{16}$$

Evaluating $u_2(75) = 0.38$ from Fig. 2B, and assuming the values k_2 and k_3 which we found to be correct, one obtains the inconsistency $(0.17) = (0.36)(0.38)$.

To correct this, there are several alterations of the responses that could lead to consistency. The simplest involves changing the indifference pairs in one of Fig. 2D, E, or H. The three possibilities are indicated in Table 2.

It was decided the first change seemed reasonable, resulting in $k_1 = 0.8$, $k_2 = 0.36$, and $k_3 = 0.14$. Using these, k_N was calculated from (9) to be -0.71. Thus from (12), the net fishermen's utility function is

$$u_N(x_1, x_2, x_3) = \frac{1}{-0.71}\left[(1 - .568u_1(x_1))\right.$$
$$\left.(1 - .256u_2(x_2))(1 - .1u_3(x_3)) - 1\right] \tag{17a}$$

where

$$u_1(x_1) = 1.26(1 - e^{-.0000633x_1}) \tag{17b}$$

[2]The certainty equivalent for a lottery is a certain amount of an attribute that is equivalent (i.e. indifferent) to that lottery. In interpreting certainty equivalents for income, it is important to realize the amounts refer to fishing income. Fishermen have other sources of income including government aid.

TABLE 2. Tradeoff changes to achieve consistency of u_N ("$\sim$" means "is indifferent to").

Change	Implications
1. k_3 from 0.17 to 0.14	$(x_1 = 9342; x_3 = 1) \sim (x_1 = 0, x_3 = 10)$ in Fig. 2E
2. k_2 from 0.36 to 0.45	$(x_1 = 2362; x_2 = 100) \sim (x_1 = 0, x_2 = 0)$ in Fig. 2D
3. $u_2(75)$ to $u_2(67.5)$ in (16)	$(x_2 = 100, x_3 = 10) \sim (x_2 = 67.5, x_3 = 1)$ in Fig. 2H

$$u_2(x_2) = 1.385 - 0.385e^{.0128x_2} \quad (17c)$$

and

$$u_3(x_3) = 1.06 - 1.458e^{-0.319x_3} \quad (17d)$$

The Lure Fishermen's Utility Function

Hilborn felt that as a first cut, the same utility function could be used for the lure fishermen as for the net fishermen. The only difference is that the income for the year and the workday measures pertain to the lure fishermen. Different policies of the DOE could have very different implications in terms of income, for instance, on the net and lure fishermen. Hence, although we have taken their preference structures to be the same, it does not follow that their preferences for options will agree. For reference the lure fishermen's utility function is

$$u_L(x_4, x_5, x_6) = \frac{1}{-0.71}[(1 - .568u_4(x_4))(1 - .256u_5(x_5))(1 - .1u_6(x_6)) - 1] \quad (18a)$$

where

$$u_4(x_4) = 1.26(1 - e^{-.0000633x_4}) \quad (18b)$$

$$u_5(x_5) = 1.385 - 0.385e^{.0128x_5} \quad (18c)$$

and

$$u_6(x_6) = 1.06 - 1.458e^{-0.319x_6} \quad (18d)$$

The Sport Fishermen's Utility Function

Here there was only one attribute, the annual catch of sport fishermen. We assessed 300,000 indifferent to a 50–50 lottery yielding 0 or 1,000,000. Using these three points indicated in Fig. 3, we fit the exponential utility function

$$u_S(x_7) = 1.198(1 - e^{-.0000018x_7}) \quad (19)$$

where x_7 is the number of fish in the annual catch. It was also checked that the utility function was indeed risk averse over the entire range.

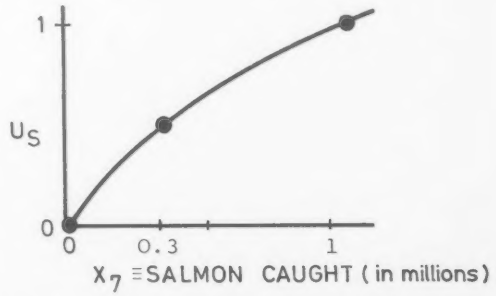

FIG. 3. Hilborn's utility function for sport fishermen.

A Utility Function for the Region

The assessments for the three attributes X_8, X_9, X_{10} to obtain the region's utility function are all indicated in Fig. 4. First the individual utility functions were assessed. An interesting fact is that $u_{10}(x_{10})$ in Fig. 4C is risk prone. This is due to the feeling that once salmon prices get above a few dollars a pound, most of the people interested in eating the fish would not buy it. Thus, one would be just willing to risk a one-half chance at \$10/lb for a one-half chance at \$0.20/lb if the alternative was \$2/lb for certain. The consistency check in Fig. 4H implies a change must be made in one of the original assessments. This was done in Fig. 4E.

The final values of the k_i's were $k_8 = .8$, $k_9 = .43$, and $k_{10} = .125$. Using (9), the additional scaling factor in the multiplicative utility function was evaluated to be -0.76. Thus the regional utility function is

$$u_R(x_8, x_9, x_{10}) = \frac{1}{-0.76}[(1 - .61u_8(x_8))(1 - .327u_9(x_9))(1 - .095u_{10}(x_{10})) - 1] \quad (20a)$$

where

$$u_8(x_8) = 1.44(1 - e^{-.000237x_8}) \quad (20b)$$

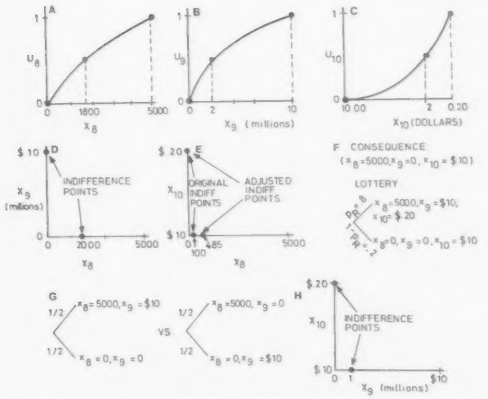

FIG. 4. Assessing Hilborn's utility function for the region.

$$u_9(x_9) = 1.04(1 - e^{-.328x_9}), \; x_9 \text{ in millions} \tag{20c}$$

and

$$u_{10}(x_{10}) = -.027 + 1.113^{-.37x_{10}} \tag{20d}$$

THE INDIANS' UTILITY FUNCTION

Hilborn felt 20,000 fish caught in a year was indifferent to the lottery yielding either 0 or 100,000 fish, each with probability one half. An exponential utility function scaled 0–1 consistent with such preferences is

$$u_I(x_{11}) = 1.04(1 - e^{-.0000328x_{11}}) \tag{21}$$

It is shown in Fig. 5.

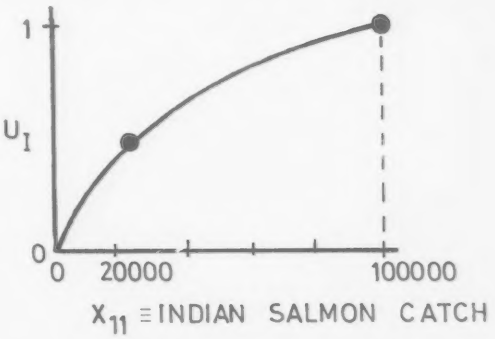

FIG. 5. Hilborn's utility function for the Indians.

5. Hilborn's Overall Utility Function for the Canadian Department of the Environment

We now move up to the top level of the objectives hierarchy in Fig. 1 and assess a utility function for DOE. Our approach consists of two steps. First, we integrate the preferences of the five groups into a single indicator of preference $u(u_N,u_L,u_S,u_R,u_I)$, and then, we combine this with attribute X_{12}, the cost to the Canadian government, to obtain an overall DOE utility function $u_G(u,x_{12})$. Notationally, for each u_j (i.e. u_N,u_L,u_S,u_R,u_I), we will create an associated attribute U_j.

INTEGRATING THE PREFERENCES OF THE INTEREST GROUPS

First, we investigated whether the assumptions necessary for Theorem 1 seemed reasonable. In this context, the preferential independence conditions imply that tradeoffs between any two groups (measured on the U_j scales) do not depend on how well satisfied the other three groups are. The utility independence conditions mean that if the consequences to only one group are uncertain, we will always choose among alternatives using only the outputs to that group.

We decided the utility independence conditions seemed reasonable. However, the preferential independence assumptions were not completely justified, since tradeoffs between the net fishermen and the region, for example, depended on the lure fisherman. If the lure fishermen were doing poorly, there was a willingness to give up more in regional development to move net fishermen from a bad to a good position (e.g. a 0.2 to a 0.8 utility measured by u_N) than there was if lure fishermen were doing well. That is, there was a premium on having at least one group of commercial fishermen at a good level.

Next the possibility of combining u_N and u_L into a commercial fishermen's utility function u_C with associated attribute U_C was investigated. Tradeoffs between U_C and U_R were examined and they did seem to be preferentially independent of $\{U_S,U_I\}$. Thus, we could use Theorem 2 to combine u_N and u_L into u_C and then use Theorem 1 to combine u_C, u_S, u_I, and u_R into u.

There is one additional assumption that seems reasonable in the group context. In terms of our problem, the assumption is that DOE's utility function for satisfying group j is group j's utility function. This assumption just says DOE's utility function for any of the five attributes U_N, U_L, U_S, U_R, and U_I is linear.

Combining this assumption with those previously verified, we find

$$u_C(u_N, u_L) = \lambda_N u_N + \lambda_L u_L + (1 - \lambda_N - \lambda_L) u_N u_L \quad (22)$$

and

$$u(u_C, u_S, u_R, u_I) = \lambda_C u_C + \lambda_S u_S + \lambda_R u_R + \lambda_I u_I \quad (23)$$

or

$$1 + \lambda u(u_C, u_S, u_R, u_I) = (1 + \lambda\lambda_C u_C) \times (1 + \lambda\lambda_S u_S)(1 + \lambda\lambda_R u_R)(1 + \lambda\lambda_I u_I) \quad (24)$$

where again the u's are all scaled zero to one and the λ_j's have the same interpretation in (23) and (24).

Given (22) through (24), one needs to assess the u_j's and λ_j's. We have already assessed the u_j's except for u_C, on the scale 0–1. Hence we needed to obtain only the λ_j, j = N, L, S, R, I, C, and u_C.

Assessing u_C

First we considered the two lotteries in Fig. 6A and the second one was preferred. This substantiates an attitude found when investigating preferential independence conditions, namely, that there was a premium on having at least one of the two groups doing well. Notice that with the second lottery, at least one of the two groups is doing fine (i.e. its utility is one), whereas with the first lottery there is one-half chance that both groups do terribly (i.e. utility equal to zero). This implies that the sum of λ_N and λ_L in (22) must be greater than 1.

Next we ask whether it would be better, given that we started from $u_N = u_L = 0$, to move u_N to 1 or u_L to 1. The response was that it was equivalent, implying $u_N = u_L$. Finally, to get a specific number for λ_N we asked for the indifference probability such that the consequence and lottery in Fig. 6B were equally desirable. The indifference probability was 0.6 which, by equating the utilities of the consequence and lottery, implies $\lambda_N = 0.6$. Thus the commercial fisherman's utility function is

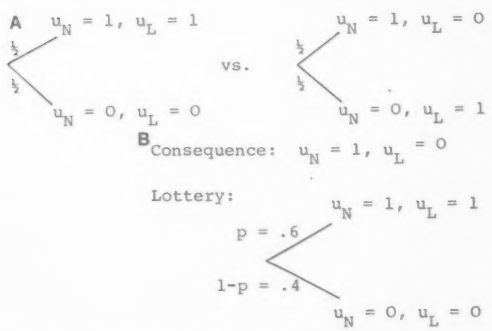

FIG. 6. Assessing Hilborn's utility function for the commercial fishermen. A, Comparing two lotteries (the second was preferred); B, Assessing p for indifference.

$$u_C(u_N, u_L) = 0.6u_N + 0.6u_L - 0.2u_N u_L \quad (25)$$

In making evaluations and tradeoffs necessary to assess u_C, we constantly made reference to the original attributes $\{X_1,X_2,X_3\}$ representing the net fisherman and $\{X_4,X_5,X_6\}$ representing the lure fisherman. Thus, for instance, to interpret $u_N = 1$, one should recall $u_N(x_1 = \$25{,}000, x_2 = 0, x_3 = 10) = 1$, and think about the implications of these amounts of X_1, X_2, and X_3. This will become clearer in what follows.

Assessing u

First we wanted to rank the λ_j's for j = S,R,I,C. This was done with the aid of Table 1. I asked, "Given that all the attributes $X_1, \ldots, X_{11}$ are at their worst level, which one of the four attribute sets $U_C \equiv \{X_1,X_2,X_3,X_4,X_5,X_6\}$, $U_S \equiv \{X_7\}$, $U_R \equiv \{X_8,X_9,X_{10}\}$ or $U_I \equiv \{X_{11}\}$ would you rather push up to its best level if you could push only one?" Hilborn chose the attributes $\{X_8,X_9,X_{10}\}$ associated with regional development, so λ_R, the regional development scaling factor, must be the largest λ_j. We then asked, "Which of the remaining three attribute sets would you prefer to move to its best level?" The answer was the commercial fisherman's attributes, implying λ_C is second largest. Continuing in the same manner led to

$$\lambda_R > \lambda_C > \lambda_I > \lambda_S \quad (26)$$

To determine values for those λ_j's, we first examined tradeoffs between U_R and U_C. However, one cannot answer questions directly in terms of u_R and u_C so we returned to the basic attributes of each group. To identify the DOE's tradeoffs, it is often best to use the attribute of each group with the largest scaling factor. The region weighted employment highest in (20), that is k_8 is greater than k_9 or k_{10}, and the commercial fishermen weighted annual income the highest. Thus we looked at the tradeoffs between X_1 and X_8 in Fig. 7A. Specifically we found ($x_1 = 0$; $x_8 = 800$) indifferent to ($x_1 = \$25{,}000$; $x_8 = 0$). Equating the utilities of these two points using either (23) or (24), while assuming the other attributes are fixed (we can assume at their worst levels because of preferential independence), yields

$$\lambda_R u_R(x_8 = 800; x_9 = 0; x_{10} = \$10) = \lambda_C \lambda_N u_N(x_1 = \$25{,}000; x_2 = 100; x_3 = 1) \quad (27)$$

Evaluating u_R with (20) and u_N with (17), we find

$$\lambda_R(.413) = \lambda_C \quad (28)$$

Next we considered tradeoffs between commercial fishermen and sport fishermen by finding the two indifference points involving X_1 and X_7 in Fig. 7B. Using the same reasoning as above, we found

$$\lambda_S = \lambda_C \lambda_N k_1 u_1(\$3{,}000) = \lambda_C(.131) \quad (29)$$

Third, DOE's tradeoffs between Indians and commercial fishermen were elaborated by assessing the indifference points in Fig. 7C. Equating utilities and using (17) and (21) led to

$$\lambda_I = \lambda_C \lambda_N k_1 u_1(\$7{,}000) = \lambda_C(.270) \quad (30)$$

Equations (28) through (30) fixed the relative values of the λ_j's. To get their absolute level, we assessed the probability P where the lottery and consequence in Fig. 7D would be indifferent. The sure consequence has the region attributes $\{X_8, X_9, \text{ and } X_{10}\}$ at their best and the attributes $\{X_1, \ldots, X_7, X_{11}\}$ of the four fishing groups at their worst. The lottery yields all 11 attributes at their best with probability P or all at their worst otherwise. The indifference probability was found to be 0.4. Equating the utility of the consequence to that of the lottery using either (23) or (24) gives

$$\lambda_R = 0.4 \quad (31)$$

The set of equations (28)–(31) was solved to yield

$$\lambda_R = 0.4, \quad \lambda_C = 0.165, \quad \lambda_I = 0.045, \quad \lambda_S = 0.022 \quad (32)$$

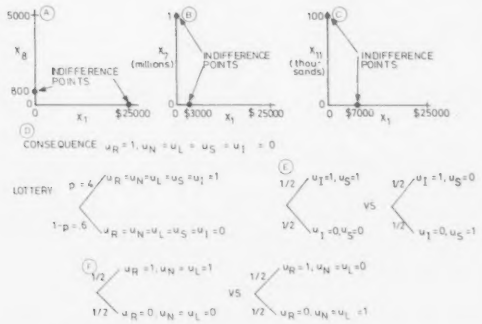

FIG. 7. Assessing Hilborn's utility function for the Skeena groups.

Since the sum of these four λ_j's is not 1, the multiplicative form (24) is appropriate. The parameter λ was calculated to be 3.05 from the λ_j's in (32). The resulting utility function is

$$u(u_C, u_S, u_R, u_I) = \frac{1}{3.05}[(1 + .503u_C) \times (1 + .067u_S)(1 + 1.22u_w)(1 + .137u_I) - 1] \quad (33)$$

where the u_j's are as specified in (19)–(21) and in (25).

Consistency Checks

As a check on the multiplicative form with $\Sigma\lambda_j < 1$, we asked which of the two lotteries in Fig. 7E was preferred. One can see that both lotteries give a one-half chance of both the sport fishermen and the Indians having either their best or worst situation. However, the first lottery has both at their best or both at their worst. The second lottery has one of the two groups at its best level with the other at its worst. For reasons of equity the first lottery was preferred, which is consistent.

As a second check, the two lotteries in Fig. 7F were compared. These have the same interpretation as above, only they lump the commercial fishermen together. Here it was also felt that the first lottery was preferred. (Note that in the real world, excellent fishing conditions correlate with high satisfaction of the regional development. However, the lotteries in 7F are not asking this question; they are concerned with preferences for the impacts specified by the lotteries.) The choice of the first lottery in 7F is consistent with the utility function (33).

Assessing u_G

To aggregate u and x_{12} into an overall utility function u_G for DOE, it seemed reasonable to assume U and X_{12} were utility independent of each other. Thus from Theorem 2,

$$u_G(u, x_{12}) = hu + h_{12}u_{12}(x_{12}) - (1 - h - h_{12}) \times uu_{12}(x_{12}) \quad (34)$$

where we have used u itself to measure the utility of specific amounts of U.

We asked which of the lotteries in Fig. 8A was preferred and found the second one was. This implied $h + h_{12}$ was greater than 1. To get values for h and h_{12}, we assessed the probabilities such that the respective consequences and lotteries in Fig. 8B and C were indifferent. These implied

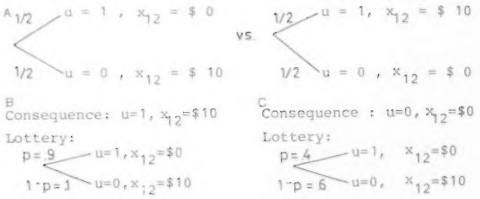

FIG. 8. Assessing Hilborn's utility function for the Canadian Department of the Environment.

that $h = .9$ and $h_{12} = .4$, so

$$u_G(u, x_{12}) = 0.9u + 0.4u_{12}(x_{12}) - 0.3uu_{12}(x_{12}) \quad (35)$$

Lastly, we needed to assess u_{12}. This was done by finding $x_{12} = \$6$ was indifferent to a lottery yielding either $x_{12} = \$0$ or $x_{12} = \$10$, each with a one-half probability. Fitting a constantly risk averse utility function to this data implies

$$u_{12}(x_{12}) = 1.784 - .784e^{.082x_{12}}, \quad x_{12} \text{ in millions of dollars} \quad (36)$$

Equations (33), (35), and (36) give us one possible overall utility function u_G for DOE.

6. Walters' First-Cut Utility Functions for the Interest Groups

In this and the next section, another preliminary utility function using C. Walters' judgments was assessed. In the discussion we will not include as much detail as in the previous case since the overall process is similar. However, the aggregation of Walters' preferences required a different structure and these differences will be illustrated. To avoid interchanging the utility functions of Hilborn and Walters, we will use v as a utility function for Walters, whereas u was used previously.

The Net Fishermen's Utility Function

We began by assessing the net fishermen's utility function using either the additive form (11) or the multiplicative form (12). The utility functions v_1, v_2, and v_3 for attributes X_1, X_2, and X_3 were first assessed. We found the certainty equivalent for the lottery <\$0;\$25,000> yielding a one-half chance at each of the two consequences was $x_1 = \$8000$. An exponential fit then resulted in the utility function shown in Fig. 9A. A similar exponential fit gave us u_3 in Fig. 9C.

Preferences for attribute X_2 were nonmonotonic. They increased from 0 to 50 days fishing and then dropped off to an absolute minimum at

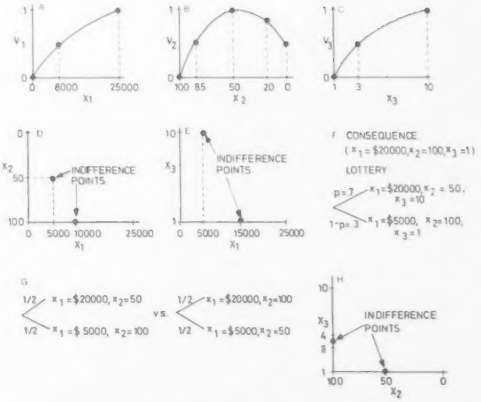

FIG. 9. Assessing Walters' utility function for the net fisherman.

100 days. As a result, two exponential fits were used for v_2. Using 85 indifferent to <50,100>, we specified v_2 for x_2 between 50 and 100, and using 20 indifferent to <0,50> we specified v_2 for x_2 between 0 and 50. To consistently scale these two parts we found 85 days and 0 days were equally desirable. Thus $v_2(0) = v_2(85)$. The final result is shown in Fig. 9B.

To evaluate the scaling factors k_1, k_2, and k_3, the indifference pairs in Fig. 9D and E were assessed along with the indifference probability in Fig. 9F. This gave us $k_1 = 0.84$, $k_2 = 0.29$, and $k_3 = 0.49$.

For two checks, we found the preferences between the lotteries of Fig. 9G and the indifference pair in 9H. Both of these were consistent. The final utility function is

$$v_N(x_1, x_2, x_3) = \frac{1}{-0.9}[(1 - .756v_1(x_1)) \times (1 - .261v_2(x_2))(1 - .441v_3(x_3)) - 1] \quad (37a)$$

where

$$v_1(x_1) = 1.26(1 - e^{-.0000633x_1}) \quad (37b)$$

$$v_2(x_2) = \begin{cases} 1.392 - 0.0892e^{-.0164x_2}, & 0 \le x_2 \le 50 \\ 1.197 - 0.033e^{.036x_2}, & 50 \le x_2 \le 100 \end{cases} \quad (37c)$$

and

$$v_3(x_3) = 1.06 - 1.458e^{-0.319x_3} \quad (37d)$$

There is one important difference about the assessment procedures used for u_N and v_N. For evaluating tradeoffs with v_N, the lowest income used was \$5,000, whereas \$0 was used in assessing u_N. Since \$0 is extremely unlikely as well as

severe, it is much more difficult to evaluate tradeoffs when possibilities of obtaining \$0 exist. The change to use "more thinkable values" likely leads to better assessments.

The Lure Fishermen's Utility Function

The procedure followed was identical to that for the net fisherman. Using the information in Fig. 10, the utility function was found to be

$$v_L(x_4, x_5, x_6) = \frac{1}{-.46}[(1 - .304v_4(x_4)) \times (1 - .147v_5(x_5))(1 - .092v_6(x_6)) - 1] \quad (38a)$$

where

$$v_4(x_4) = 1.01(1 - e^{-0.170x_4}) \quad (38b)$$

$$v_5(x_5) = \begin{cases} 1.234(1 - e^{-0.021x_5}), & 0 \le x_5 \le 80 \\ 1.078 - 0.0029e^{0.041x_5}, & 80 \le x_5 \le 100 \end{cases} \quad (38c)$$

and

$$v_6(x_6) = 1.06 - 1.458e^{-0.319x_6} \quad (38d)$$

The scaling factors were $k_4 = 0.66$, $k_5 = 0.32$, and $k_6 = 0.20$.

Let us briefly comment on two differences between the lure and net fishermen's utility functions. First, the lure fishermen are more risk averse in the monetary attribute. This is mainly because they work most of the year and have little chance to subsidize their fishing incomes. The net fishermen, however, have a season and can recover from a bad fishing year more easily by working during that part of the year when they are not fishing. The other main difference is in the lure and net fishermen's utility functions for days worked. To see this compare Fig. 9B and 10B. The lure fishermen work mainly in the daytime in pleasant weather whereas the net fishermen must fish at night in all kinds of weather during the season.

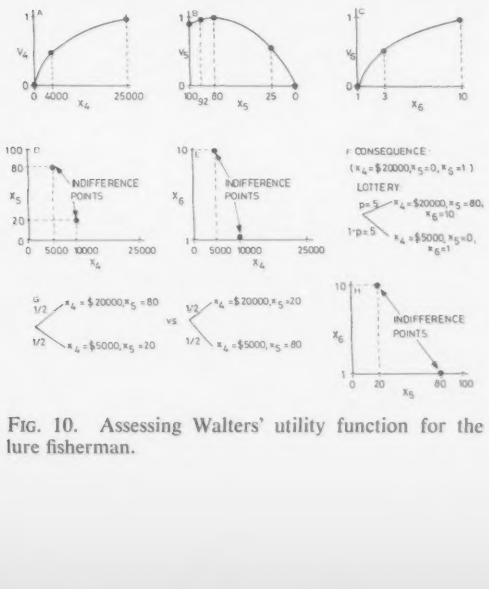

FIG. 10. Assessing Walters' utility function for the lure fisherman.

The Sport Fishermen's Utility Function

The certainty equivalent for the 50–50 lottery yielding 0 or 1,000,000 was 200,000. Fitting an exponential utility function to this, we found

$$v_S(x_7) = 1.039(1 - e^{-.00000328x_7}) \quad (39)$$

where x_7 is the number of fish in the annual catch. This utility function is illustrated in Fig. 11.

The Utility Function for the Region

The information used to assess the utility function for the region is illustrated in Fig. 12. This was done exactly as in previous cases except for the fact that the lottery used to assess v_8 did not cover the entire range. That is, we did not use the certainty equivalent to the lottery $<0;5,000>$. Rather, we found 2,000 indifferent to $<1,000; 5000>$ which avoids the extra difficulty of thinking about the extreme of 0 employment.

The scaling factors were $k_8 = 0.81$, $k_9 = 0.34$, and $k_{10} = 0.07$ implying $k = -0.64$, and the utility function was

$$v_R(x_8, x_9, x_{10}) = \frac{1}{-0.64}[(1 - 0.52v_8(x_8)) \times (1 - 0.22v_9(x_9))(1 - 0.044v_{10}(x_{10})) - 1] \quad (40a)$$

where

$$v_8(x_8) = 1.05(1 - e^{-.000609x_8}) \quad (40b)$$

$$v_9(x_9) = 1.01(1 - e^{-0.455x_9}) \quad (40c)$$

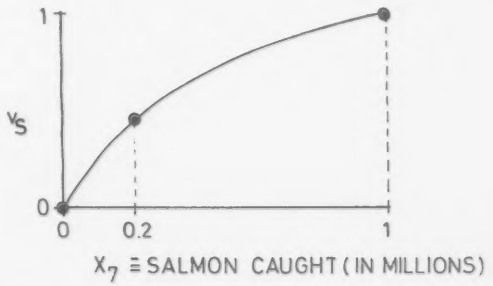

FIG. 11. Walters' utility function for the sport fisherman.

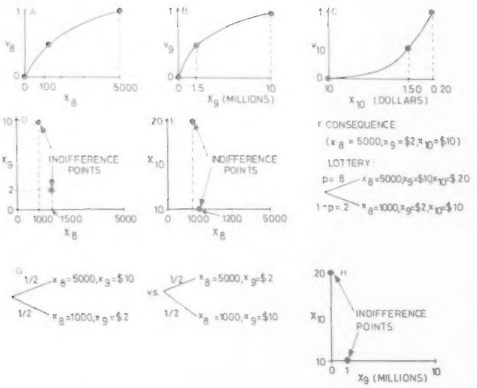

FIG. 12. Assessing Walters' utility function for the region.

and

$$v_{10}(x_{10}) = -5.64 + 1.118e^{-0.529x_{10}} \quad (40d)$$

THE INDIANS' UTILITY FUNCTION

We found 10,000 was indifferent to a lottery yielding either 0 or 100,000 each with probability of one half. Fitting this, we find (Fig. 13):

$$v_I(x_{11}) = 1.001(1 - e^{-.0000692x_{11}}) \quad (41)$$

7. Walters' Overall Utility Function for the Canadian Department of the Environment

Having assessed the five groups' utility functions, we can consider DOE's problem to aggregate six attributes: V_N, V_L, V_S, V_R, V_I, and X_{12}, which are measured by v_N, v_L, v_S, v_R, v_I, and x_{12}, respectively.

THE ASSUMPTIONS

First we examined the assumptions necessary to directly invoke Theorem 1 for these six attributes. For instance, one check used the four pairs of lotteries in Fig. 14. The first of each of these pairs offers either both of the attributes at their best levels or both at their worst, whereas the second lottery in each pair has one attribute at the best level and the other at the worst level for each of the two possible consequences. In all cases, the second lottery was preferred indicating a strong tendency to "do well in terms of at least one attribute." Had it not been the case that either the first or the second lottery was preferred for all four sets, then the preferential independence assumptions necessary for Theorem 1 would have been violated. Direct examination of

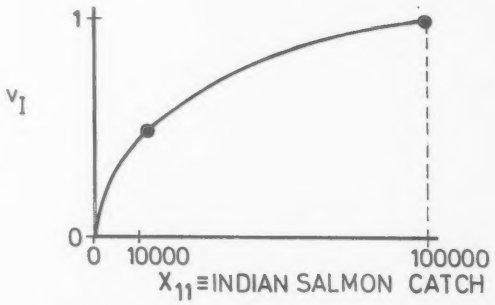

FIG. 13. Walters' utility function for the Indians.

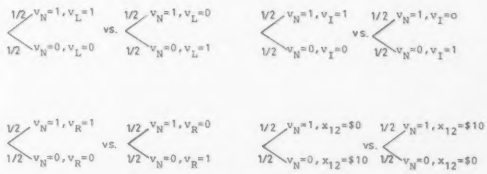

FIG. 14. Comparing lotteries (the second was always preferred).

some tradeoffs led us to conclude the assumptions did hold and that we could use Theorem 1.

THE ASSESSMENTS

Since we had already assessed all the necessary component utility functions except for v_{12}, the remaining task was to do this and then determine the scaling constants for the overall additive or multiplicative utility function.

A different range, zero to fifty million dollars, was used on assessing v_{12} than on the corresponding u_{12} in Hilborn's utility function. Walters felt 25 was indifferent to the lottery $<0,50>$ and that the utility function should be linear. It is

$$v_{12}(x_{12}) = 1 - 0.02x_{12},\ x_{12} \text{ in millions} \quad (42)$$

To assess the scaling constants, tradeoffs between X_8 and an attribute within each of the other five groups of attributes were examined. The pairs of indifference points are shown in Fig. 15A–E. Equating the utilities of the indifference points gives us five equations with six unknowns, the scaling constants for V_N, V_L, V_S, V_R, V_I, and X_{12}. A sixth equation is provided by assessing the indifference probability in Fig. 15F. Such a high indifference probability again indicates the strong desire to have at least some measures look good.

The scaling constants were calculated to be $\lambda_N = 0.76$, $\lambda_L = 0.9$, $\lambda_S = 0.57$, $\lambda_R = 0.9$, $\lambda_I = 0.24$, and $\lambda_{12} = 0.66$ implying $\lambda = -0.999$. The final DOE utility function is

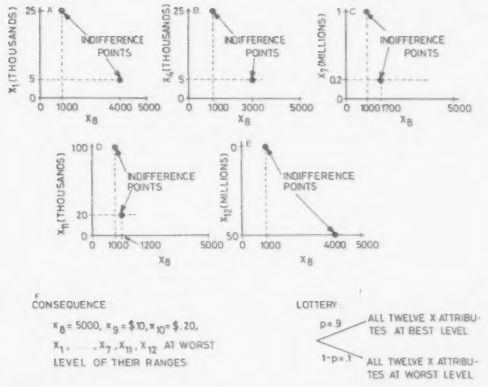
INDIFFERENCE POINTS
INDIFFERENCE POINTS
INDIFFERENCE POINTS
INDIFFERENCE POINTS
INDIFFERENCE POINTS
CONSEQUENCE
x_8 = 5000, x_9 = $10, x_{10} = $.20, x_1, ..., x_7, x_{11}, x_{12} AT WORST LEVEL OF THEIR RANGES
LOTTERY
p=.9
ALL TWELVE X ATTRIBUTES AT BEST LEVEL
1-p=.1
ALL TWELVE X ATTRIBUTES AT WORST LEVEL

FIG. 15. Assessing Walters' utility function for the Canadian Department of the Environment.

$$v_G(v_N, v_L, v_S, v_R, v_I, x_{12}) = \frac{1}{-0.999}[(1 - .76v_N) \times (1 - .9v_L)(1 - .57v_S)(1 - .9v_R)(1 - .24v_I) \times (1 - .66v_{12}(x_{12})) - 1] \quad (43)$$

where v_N, v_L, v_S, v_R, v_I, and v_{12} are defined by (37) through (42), respectively.

8. Comparing the Two Overall Utility Functions

In this section, we will examine briefly some of the similarities and differences implied by Hilborn's utility funtcion u_G and Walters' utility function v_G. The purpose is twofold: 1) to illustrate the wide variety of preference attitudes which can be captured by multiattribute utility functions, and 2) to set the stage for suggestions for further work. In interpreting these comparisons, two things should be kept in mind. First, there are no objectively right or wrong utility functions. These functions formalize subjective preferences and different individuals can (and do) have different preferences. Second, both Hilborn's and Walters' utility functions are first-cut utility functions. Although we did do some preliminary consistency checks during the assessments, we have not had the opportunity for in-depth examinations needed to obtain a final representation that captures all of one's basic preference attitudes. With these caveats, let us first look at the various groups' utility functions, and then at the overall preferences of DOE.

The Net Fishermen's Utility Functions

Hilborn's utility function for the net fisherman is given in Fig. 2 and Walters' in Fig. 9. One immediately sees that u_1 and v_1 and also u_3 and v_3 are identical. However, u_2 and v_2 are different. With u_2, the less one works, assuming constant income as measured by attribute X_1, the better. With v_2, preferences increase up to a maximum at 50 days and then decrease. This implies, for instance, that a net fisherman would be willing to give up some amount of income to increase fishing time from 20 to 50 days. The function u_2 implies a dislike for fishing per se, whereas v_2 implies that there is a positive value in itself to fish as long as it is not too much fishing.

For the tradeoffs, we can look at the values assigned to k_1, k_2, and k_3. Hilborn's preferences implied $k_1 = 0.8$, $k_2 = 0.36$, and $k_3 = 0.17$, and Walters' were $k_1 = 0.84$, $k_2 = 0.29$, and $k_3 = 0.49$. The major difference here is in the weighting of k_3. Walters considered it much more important relative to net fishermen's incomes, to keep X_3 high. As one indication, Hilborn would allow X_3 to drop from 10 to 1 to have X_1 increase from 0 to \$2,400. To accept this same decrease in X_3, Walters required income to increase from 0 to \$9,800.

The Lure Fishermen's Utility Functions

Recall that Hilborn used the same utility functions for lure and net fishermen. Hence, to compare Hilborn's and Walters' lure fishermen's utility functions, refer to Fig. 2 and 10. The utility functions u_6 and v_6 are identical. For income, v_4 is more risk averse than u_4. As explained in Section 5, Walters felt the lure fishermen would be more risk averse because they fish over the entire year and have little chance to subsidize their income by other activities. The utility functions u_5 and v_5 are quite different for the same reason as discussed for the net fisherman.

The scaling constants are $k_4 = 0.8$, $k_5 = 0.36$, and $k_6 = 0.17$ for Hilborn and $k_4 = 0.66$, $k_5 = 0.32$, and $k_6 = 0.20$ for Walters. The relative values here are about the same, implying one would be willing to give up, for example, roughly the same in income (as measured by utility for income) to increase a fixed degree in either workdays or variety (as measured by their respective utility functions).

Hilborn and Walters prefer a lottery which has one of two attributes at a desirable level to a lottery with either both at a desirable or undesirable level (Fig. 2G and 10G). Such an attitude implies $k_4 + k_5 + k_6 > 1$.

The Sport Fishermen's Utility Function

Comparing Fig. 3 and 11, we see v_8 is a little

more risk averse than u_S. Hilborn was indifferent between 0.3 million fish caught for sure or a 50–50 chance at 0 or 1 million, whereas Walters preferred 0.3 million to the lottery. However, Walters was indifferent between 0.2 million for sure and a 50–50 chance at 0 or 1 million. Hilborn would choose the lottery in this last situation. Both are risk averse, however, and the differences would likely not be critical for policy decisions. Of course, this could be checked in any evaluation of potential policies.

The Region's Utility Function

The three component utility functions for the region: u_8, u_9, and u_{10} and v_8, v_9, and v_{10}, respectively, are similar to each other. This can be seen by comparing Fig. 4 and 12. The only real difference seems to be that 1800 is indifferent to the lottery <0;5,000> using u_8 whereas 1100 is indifferent using v_8. Both utility functions for employment and both for recreational income are risk averse, whereas those for salmon price are risk prone.

The scaling factors for u_R are $k_8 = 0.8$, $k_9 = 0.43$, and $k_{10} = 0.125$ and for v_R and $k_8 = 0.81$, $k_9 = 0.34$, and $k_{10} = 0.07$. These two sets have similar implications. The differences in the k_{10} result in a big difference in tradeoffs between X_8 and X_{10}. Hilborn would allow the price of salmon to rise from \$.20 to \$10 only if employment increased from 0 to 485. That is ($x_8 = 0$, $x_{10} =$ \$.20) is indifferent to ($x_8 = 485$, $x_{10} =$ \$10). However, Walters would allow the same price increase to raise employment to 140. This means for him ($x_8 = 0$, $x_{10} =$ \$.20) is indifferent to ($x_8 = 140$, $x_{10} =$ \$10).

Again, by comparing part G of Fig. 4 and 12, one sees both individuals felt it was better to do well by at least one measure than risk a one-half chance of a poor performance on both.

The Indians' Utility Function

Both Hilborn and Walters assessed risk averse utility functions for the Indians. They would be willing to give up significant chances of a big catch to insure some small catch. Hilborn felt 20,000 was indifferent to a 50–50 chance at 0 or 100,000, whereas Walters felt this indifference point was 10,000. The utility functions u_1 and v_1 are illustrated in Fig. 5 and 13, respectively.

The Overall DOE Function

Recall that the methods of aggregating the component utility functions into an overall utility function were different for Hilborn and Walters. Hilborn required that one "nest" three levels of multiplicative utility functions, whereas one level was required for Walters. The additional levels provide for more complicated interaction of preferences among the groups.

From Fig. 14, one can see that for Walters, whenever two lotteries were given which had the same possible outcomes when viewed one attribute at a time, the lottery that guaranteed at least one attribute to be at a desirable level was preferred. This was not always the case with Hilborn. As seen from Fig. 6A, it was the case if the two attributes indicated respectively the net and lure fishermen's interests.

However, considering the four groups — commercial fishermen, sport fishermen, the region, the Indians — the situation altered for Hilborn (Fig. 7E and F). Hilborn preferred the first lottery in each case, reasoning that both outcomes of the second lottery were not equitable — one group always did well, but at the expense of the other. In the first lottery, at least the groups were "in the same boat." This attitude implied the scaling factors λ_R, λ_C, λ_I, and λ_S in (32) need to sum to less than one. This attitude is not shared by Walters (Fig. 14). He still prefers to have some group doing well and is willing to give up a little in "equity" to achieve this.

When one gets down to possible combinations of group satisfaction and DOE costs, Hilborn and Walters are in general agreement again. Compare the last lottery pair in Fig. 14 with Fig. 8A. In both cases the second lottery was preferred, because one could perhaps justify either spending a lot to get many desirable benefits or spending nothing to get few benefits. However, there is a chance in the first lottery of spending a lot and getting nothing in return. That combination is particularly undesirable.

If one looks at the scaling factors of u_G and v_G, a direct comparison is not possible because the functional forms are different. However, we can look at the utility of having attribute U_j (or V_j) at its best level while all other attributes are at their worst levels; call this $\bar{u}_j$ (or $\bar{v}_j$), measured relative to the scale of u_G (or v_G) from 0 to 1. Doing this, we find $\bar{u}_N = 0.09$, $\bar{u}_L = 0.09$, $\bar{u}_S = 0.02$, $\bar{u}_R = 0.36$, and $\bar{u}_I = 0.04$ and $\bar{v}_N = 0.76$, $\bar{v}_L = 0.9$, $\bar{v}_S = 0.57$, $\bar{v}_R = 0.9$, and $\bar{v}_I = 0.24$. This is a major difference mainly due to the differences among preferences for lottery pairs discussed earlier. It means that the indifference probability for the lottery in Fig. 15F using Hilborn's preferences would be 0.36 rather than 0.9 as was the case with Walters.

The utility functions for the expenses of DOE, attribute X_{12}, are different. Walters felt prefer-

ences should be linear, implying an expenditure of $5 million indifferent to a 50–50 chance of 0 or 10. Hilborn felt 6 million was indifferent to <0,10>.

9. Use of Utility Analysis of the Skeena Problem

To date, we have made only rough assessments of utility functions for two individuals knowledgeable about Skeena River policy options. What are some of the possible uses of these? One is to more clearly articulate substantive issues of the problem and sensitize various individuals to these issues. For instance, some of the basic preference attitudes indicated by u_G and v_G may be critical in evaluating policy options. To identify these as important should lead to more concrete discussion of these aspects. Note that this is true whether or not the final choice of policy will be made assisted by a utility model (or any model) or by an in-the-head evaluation.

By addressing the preference issues, some ambiguities in the problem structure often appear. For instance, it may become apparent that difficulties in assessment are due to double counting — for example, does one prefer more fishing days only for the income it brings or for the fishing itself? Having identified such difficulties, they can often be clarified.

The individual utility functions aid communication among members of a team working on a problem. They should help to isolate differences in judgments about possible impacts of a policy from differences in preferences for these impacts. This could be an important step toward resolving these differences.

It may be possible for the different members of the study team to come to some agreement on a reasonable utility function for evaluating Skeena development. To do this would require detailed examination of the implications of the utility function to make sure it does imply all preference attitudes that seem relevant. This would involve significant interaction among the group members, as well as with outside experts including members of the groups involved in the problem. If it is not possible for the team members to agree on one utility function, they can use maybe two or three and examine differences in implications for policy.

Given we do have utility functions, they can be used to aid in examining and evaluating policies. For this they would need to be used in conjunction with the dynamic model that generates the impacts of various possible policies. Suppose one option is fine for all the groups but one. By investigating why this group fares so poorly in terms of its component attributes, we may find a hint toward generating a new alternative which raises that "low" group significantly at little expense to the other groups.

If there is no agreement on an overall utility function, perhaps each of the possibilities will lead to the same "best" options; or at least each utility function may imply that some options can be discarded from future consideration.

Possible Future Work

Four general categories of research should be considered. The first is to carefully assess a (or two or three) utility function(s) representing the teams' understanding of the preferences. Second, it would be interesting to directly assess utility functions of various members in the groups of net fishermen, lure fishermen, Indians, DOE, and other individuals concerned about the Skeena. Some initial work on this aspect is discussed in Hilborn and Walters (1977). These utility functions could be used to refine an overall government utility function. Third, it would be important to try to account for the temporal aspects of the problem. Certainly, any major policy adopted now would likely have impact for at least a few years to come. At present, the variety attributes are proxy variables for "the future," but this is not really adequate. Along this line, some recent work of Bell (1975) concerning time preferences for evaluating control strategies for the spruce budworn, a forest pest, may prove helpful. As a final suggestion, I feel it would be worthwhile to begin examining possible policies with the utility model. It certainly is not perfect but the alternative is to somehow aggregate all those output data in one's head — and that's not perfect either.

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Differing Goals of Salmon Management on the Skeena River

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HILBORN, R., AND C. J. WALTERS. 1977. Differing goals of salmon management on the Skeena River. J. Fish. Res. Board Can. 34: 64–72.

In this paper we describe the use of a specific decision analysis tool, multiattribute utility analysis, to investigate conflicting goals in the management of salmon on the Skeena River. We used this technique to determine the preferences of 10 individuals representing several interest groups and different management agencies. It is shown how individuals differ in their preferences, how conflicts can be identified, and how decision analysis can be used to refine an individual's understanding of his preferences. Individuals assessed possible outcomes of different alternative enhancement proposals using two techniques, the decision analysis technique and an intuitive approach. These two methods produced different results. The use of these techniques in the management process is discussed.

HILBORN, R., AND C. J. WALTERS. 1977. Differing goals of salmon management on the Skeena River. J. Fish. Res. Board Can. 34: 64–72.

Nous décrivons dans cet article l'emploi d'un instrument spécifique d'analyse de décisions, l'analyse d'utilité à multiples attributs, dans l'examen d'objectifs contradictoires dans la gestion du saumon sur la rivière Skeena. Nous nous sommes servis de cette technique pour déterminer les préférences de 10 individus représentant plusieurs groupes d'intérêt et différents organismes de gestion. On y démontre comment les individus diffèrent dans leurs préférences, comment les conflits peuvent être identifiés et comment l'analyse des décisions peut donner à un individu une compréhension plus raffinée de ses préférences. Les individus ont évalué les conséquences possibles de propositions alternatives de rehaussement des stocks, utilisant à cette fin deux techniques, la technique d'analyse de décisions et une approche intuitive. Ces deux méthodes produisent des résultats très différents. Nous examinons l'emploi de ces techniques dans le processus de gestion.

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IN recent years there has been a growing conviction that the concept of maximum sustained yield is not the best objective for the management of most fisheries (see Roedel 1975 for examples). Management agencies recognize that there are many interest groups affected by management practices and that there are frequently conflicts between these interest groups. Simple goals such as maximum sustained yield or maximum economic value may not be most appropriate. This raised two questions: what really are the preferences of the different interest groups affected by the fishery, and to what extent should the different interest groups' desires be considered by the management agency?

A colleague of ours, R. Keeney, pointed out that this problem is not uncommon. Vast sums of money and man-years are spent trying to predict the outcomes of possible management options in many fields; yet usually very little effort is devoted to determining what the actual goals are for the manager. Fisheries management agencies do worry about human perceptions as well as fish biology, and they do employ people to try to determine how the public perceives the benefits of the fisheries. However, when specific questions are asked, such as "would fishermen prefer a lower variation in annual catch to the current high variation?" there usually are no data available to answer the question. The general response is that these problems are too complex or that these are value judgments with which no one should tamper. However, there is a large body of literature to show that complex problems can be reduced to a manageable size for decision analysis

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(Keeney and Raiffa 1976). Although value judgments certainly are involved, they will have to be made in any case, and it is probably better that they are made explicitly rather than being implicitly buried in a decision.

We had been extensively involved in simulation of fish dynamics (Walters 1975a) and many of our efforts, particularly optimization, were stymied due to poor understanding of management goals. We therefore sponsored a 2-day workshop to bring together representatives of the fishing industry, the provincial fish and wildlife branch, and the Fisheries and Marine Service to discuss the goals of management of all anadromous fishes on the Skeena River. The focus of the meeting was the use of a specific decision tool known as multiattribute utility analysis (Keeney 1977, this issue). We found in our earlier work (Walters 1975b; Walters et al. 1976) that simulation modeling is an excellent format for promoting discussion of the biological and physical dynamics of a system, and we hoped that the multiattribute utility analysis format would provide a similar catalyst for discussion of management goals.

In this paper, we describe the use of multiattribute utility analysis to determine the preferences of different individuals involved in the management of the Skeena River. We compare this quantitative method to some alternative qualitative ones.

In an idealistic world, we would like to determine objective functions on which to base our management. An objective function is a quantitative expression of the goals of management and the term applied to some "value" being optimized by management acts. However, we recognize there are always several interest groups, whose interests need to be balanced. As a first step we feel it is necessary to derive the objectives of these different interest groups. As an approximation to this, we sought to determine the preferences of several people representing different points of view. Let us define preferences as any individual's objectives. This involves the individual's willingness to trade off amounts of one attribute for amounts of another attribute. Multiattribute utility analysis seeks to find an objective or utility function that simultaneously considers several attributes in terms of preference tradeoffs. By examining preferences of several different people, we hope at least to see how sensitive people's preferences are to different possible management alternatives. We must also remember that people's preferences may change and we would like to avoid policies that are sensitive to changes in preferences.

The first morning of the workshop was spent discussing the nature of multiattribute utility analysis. We then began to analyze a specific case system, the Skeena River. This case was chosen because it is a relatively small, self-contained system, little affected by outside fisheries, yet it is an important component of the West Coast salmon fisheries and suffers from most of the problems associated with salmon management. Also, we had done a considerable amount of modeling work on the system through earlier workshops. We strongly hoped that any discussion arising from the workshop would not apply to the Skeena River only, but would have relevance to the problem of enhancement for all West Coast fisheries. The problem was divided into a number of discrete steps in the following order:

1. Identification of all interest groups affected by the fishery.
2. Identification of the indicators or quantitative factors with which each interest group would be concerned.
3. Reduction of items 1 and 2 to a small number of indicators of the dominant interest groups.
4. Selection of four possible alternative enhancement policies on the Skeena River.
5. Estimation of likely effects of the different enhancement policies on the specified indicators.
6. Estimation of likely types of "failures" of the enhancement proposals, the probabilities of these failures, and the expected impact of the failure on the indicators.
7. An "intuitive" ranking of different outcomes.
8. Assessment of each person's utilities for each indicator.
9. Assessment of each person's tradeoffs between indicators.
10. Construction of each person's utility functions from the assessment and quantitative ranking of different enhancement proposals based on the preference functions.
11. Discussion of the differences between the "intuitive" and the quantitative rankings.

The basis of this paper is the presentation of the methods and results of each step.

Identification of Interest Groups

The first step in our analysis was to identify all groups of people with similar desires who are affected by the Skeena River salmon fishery. These were called interest or pressure groups. We wanted to be as exhaustive as possible, so we considered groups as powerful as the commercial fishermen and as diffusely defined as "other Canadians." Table 1 lists all the interest groups identified.

TABLE 1. Interest groups and indicators considered in determining management strategy for Skeena River salmon.

1. Fishermen, commercial
   - $ Revenue
   - Days/week work
   - Length of season
   - Diversity of catch
   - Bulk of catch
   - Number of fishermen
   - Run timing distribution

2. Indians, aboriginal
   - Total catch
   - Diversity of catch
   - Average size
   - Location

3. Packing companies
   - Percent capacity
   - Quality
   - Species diversity
   - Size
   - Run timing
   - Profit

4. Prince Rupert commercial residents
   - Incomes
   - Length of season
   - Days per week
   - Number of fishermen
   - Opportunity cost

5. Sport fishermen
   - Size of run
   - Size of fish
   - Species diversity
   - Access
   - Number of fishermen – local
     – nonlocal
   - Facilities and services
   - Location and setting
   - Gear
   - Expectations
   - Time of year
   - Alternative activities
   - License cost
   - Cost of fishing
   - End use, CPUE

6. Management
   - Number of fishermen (fleet size)
   - $ income of fishermen
   - Catching power/boat
   - Total catch
   - Diversity of catch
   - Fish quality
   - Predictability
   - Public availability

7. Regional population
   - Size of run
   - Number of fishermen
   - Nonconsumptive use
   - Educational opportunities
   - Latent use

TABLE 1 (*Concluded*)

8. Sports outfitters
   - Number of fishermen – local
     – nonlocal
   - Latent use
   - The rest is the same as sport fishermen

9. Fish eaters
   - Price
   - Quality
   - Species
   - Availability of fresh fish through time

10. Canadians
    - Revenue
    - Quality
    - Species
    - Availability of fresh fish through time

11. Conservationists
    - Total run
    - Species diversity
    - Habitat

12. B.C. hydro
13. Port authority
14. Industrial development

    (Investment in salmon Artificial enhancement)

15. Complementary industry
    - Same as Prince Rupert commercial and sport fishermen
    - Technological development

Identification of Indicators

The second step was to identify the attributes of the fishery the interest groups would need to know to determine which of any two management outcomes they would prefer. These indicators are listed in Table 1.

Reduction of Indicators

Having listed the interest groups and their indicators, we selected a subset of six indicators that provided the most information to the workshop participants about the state of the fishery as they believed it would be perceived by the interest groups. We were greatly constrained in the time allotted for discussion and felt that by choosing only the six most important indicators we would probably cover most of the attributes in which any individual was interested. As we will see later, the most important indicator for any individual was usually considered at least 10 times as important as the least important, so that one more indicator would have added little to our understanding of people's preferences.

The six indicators selected were: 1) cost of management — this includes operating expenses and amortized cost of physical enhancement facilities; 2) average annual value of the commercial catch; 3) catch by Indian fishermen on their aboriginal fishing grounds; 4) catch by sports fishermen of traditional sports species (steelhead, chinook); 5) total number of fish in the run — this indicator was selected as a psychological indication of the "health" of the fishery; we did not want it to represent the value of the catch; and 6) total net return to the Skeena region from the commercial fishery. For all of the above indicators we were considering an annual average value over a 20-yr horizon.

At this point in the analysis it is important to explicitly describe the attributes each indicator was chosen to represent. The cost of management represents the federal government's costs in the fishery and is indirectly an indicator of the level of government commitment to the system. Initially, some participants did not consider this an important indicator, but when we performed the quantitative analysis, most participants ended up considering it one of the most important. The commercial value of the catch is an indicator of the dollar value of the fishery and represents the economic input to B.C. The direct input to the local Prince Rupert economy is covered by a separate indicator. Indian catch is used to represent the catch of the native people on the upstream fishing grounds where fish are caught only for food. The sports catch represents the interests of the sports fishermen, both local and nonresident. The total run size was selected to represent the noncommercial aspects of the health of the fishery. An ardent conservationist might prefer any management policy that insured big runs regardless of the harvests. Net economic return to the Skeena region was chosen to represent the impact of the fishery on the local economy. It was considered necessary to distinguish between total commercial value of the catch and local input because of an increasing trend towards mobility of the fishing fleet which could lead to a smaller share of the economic benefits of the fishery's reaching the local residents.

Selection of Enhancement Proposals

Since a stated goal of our preference analysis was to use multiattribute utility analysis to evaluate individual preferences for alternative enhancement policies, we arbitrarily selected four enhancement schemes for consideration. The criterion for selection was that we wished to span as wide a range of enhancement alternatives as was possible. The purpose was not to evaluate actual possibilities but to consider preferences across a wide range of outcomes. The four enhancement possibilities we considered were:

1. No further enhancement, maintain current management policies and facilities;
2. Full scale enhancement of net-caught species (sockeye and pink). This would be done with several large spawning channels (similar to the channels currently being operated on the Fulton and Pinkett rivers emptying into Babine Lake in the Skeena system);
3. Large-scale enhancement of sports species (steelhead and chinook) through construction of two large hatcheries;
4. Enhancement of all stocks using low technology, small-scale development such as small hatcheries, incubation boxes, stream clearance, etc.

Forecasts of Policy Outcome

The brief descriptions of the enhancement alternatives given in the previous section are not sufficient to evaluate alternatives, either qualitatively or quantitatively. Our next step was to specify the likely consequences of these alternatives. This is a large step. In a formal policy analysis this would consume 60–90% of the effort. However, for the purpose of this workshop we needed to specify only *some* consequences of the enhancement alternatives and to evaluate those outcomes. We were interested in how people rated the outcomes of the application of different policies, not the underlying policies. Therefore, the numbers we assign to the different indicators for each enhancement policy are significant only in that participants were asked to evaluate those numbers. Table 2 shows the expected values of the six indicators for each enhancement proposal. Within each policy–indicator box there is a top and a bottom section. The top section contains the expected annual value of that indicator if the enhancement facilities operate as expected. The bottom section contains the estimated annual value if there is a "failure" of the enhancement facilities to operate as expected.

Estimation of Failures

As fisheries personnel are well aware, many facilities do not operate as expected. There may be large-scale technical problems resulting in major fish kills, or there may be more subtle problems such as the smolts from one hatchery eating the smolts from another hatchery. Whatever the source of the "failure," we wished to explicitly assign some probability to the events for which enhancement facilities do not operate as expected. We therefore considered for each enhancement alternative the kinds of things that

TABLE 2. Intuitive estimates of outcomes of different enhancement alternatives. Top is outcome when all goes well; bottom half is outcome if "failure" occurs. The numbers to the left of the rows are the probabilities of failure.

Policy \ Indicator:		Additional cost of management	Commercial revenue	Indian catch (no.)	Traditional sports catch (no.)	Total run size (no.)	Inputs to regional economy
		0	$\$18\times10^6$	30,000	30,000	4×10^6	$\$3\times10^6$
full net	.9	$\$15\times10^6$	$\$45\times10^6$	40,000	25,000	10×10^6	$\$4\times10^6$
sports (coho & chinook)	.1	$\$16\times10^6$	$\$13\times10^6$	30,000	20,000	3×10^6	$\$2.5\times10^6$
	.8	$\$20\times10^6$	$\$40\times10^6$	60,000	70,000	6×10^6	$\$6\times10^6$
all stocks (small-scale)	.2	$\$21\times10^6$	$\$17\times10^6$	30,000	30,000	4×10^6	$\$2.5\times10^6$
	.95	$\$8\times10^6$	$\$30\times10^6$	50,000	60,000	8×10^6	$\$5.5\times10^6$
	.05	$\$8\times10^6$	$\$21\times10^6$	45,000	55,000	5×10^6	$\$5\times10^6$

could go wrong, how likely these were, and what the expected consequences of such "failures" would be. Again, this would be a complex problem in a realistic policy analysis. The workshop participants arbitrarily specified that the no-change enhancement policy was not subject to failure. Under full net enhancement, the failure was thought to consist either of a mechanical problem with the spawning channels (such as freezing), or a biological problem such as disease, or simply a failure of additional smolts to yield additional adults. Whatever the cause of the failure, we subjectively set the probability of such a problem occurring at 10% and said that it would cause the management costs to increase slightly and all other indicators to decrease to a little below what they had been before enhancement was begun. The justification for this was that if the enhancement facilities had worked well for several years, the natural stocks would likely be at least slightly reduced. When the enhancement facilities failed, the natural stocks would not be so large as they had been before enhancement was begun.

The failure considered for the sports fishery hatcheries was total failure of output in a given year. The result was considered to be a return to the preenhancement conditions for all indicators except commercial revenue and regional economic input. These were thought to be lower than before enhancement because of larger capacities in the industry and subsequent lower efficiencies.

The failure considered for the diverse all-stocks enhancement was the failure of several facilities in a given year. The probability of this was judged to be small (5%) and its impacts were also felt to be small, only a 10–20% loss in the production facilities. Table 2 summarizes in the lower and upper boxes the expected outcomes of the four enhancement policies. We must stress that this table was developed in just 1 h, without any numerical resources. We were evaluating the specified outcomes, not the underlying policies.

Intuitive Assessment of Outcomes

After filling in Table 2, each participant was asked to rank outcomes in order of preference. By ranking outcomes on an ordinal scale, we implied that the magnitude of the difference between outcomes was not of concern, only the rank order.

Assessment of Utilities for Individual Indicators

We are omitting the technical details of multiattribute utility analysis. They are contained in Keeney (1977).

This step began the formal utility assessment. The purpose was to establish a preference curve for each variable or indicator, assuming that all other variables were held constant. We arbitrarily assigned the worst case a value of 0 and the best case a value of 1. The lowest value considered for each indicator was the lowest value appearing anywhere in Table 2; the highest value considered was the highest value found in Table 2. For all indicators, except cost of management, the highest value was always preferred to the lowest value. Also, we wanted to represent the fact that preference curves are generally not linear: for instance, most participants said they thought that adding a million dollars to the commercial revenue was more valuable when commercial revenue was low than when it was high.

It is a common feature of preference curves that the added satisfaction gained by adding a unit of indicator when the indicator is high is either greater or smaller than the satisfaction derived by adding a unit of indicator when it is low.

The questioning procedure used to derive these curves is described in detail in Keeney (1977). Briefly, it can be shown that if an individual is indifferent to two choices, then the two choices have the same preference value, or utility, for that individual. For example, we asked each participant if he would prefer a commercial return of \$27 million to a 50–50 chance of either \$13 million or \$45 million. All participants agreed they would prefer the guaranteed \$27 million to the 50–50 lottery, which means that \$27 million has a higher preference than the average value of the two choices in the lottery. After several iterations we were able to establish for each participant the exact commercial revenue for which he was indifferent to the lottery. Assume the indifference point was \$20 million, then:

$$U(\$20 \text{ million}) = .5\,U(\$45 \text{ million}) + .5\,U(\$13 \text{ million})$$

$$U(\$20 \text{ million}) = .5(1.0) + .5(0)$$

$$U(\$20 \text{ million}) = .5$$

It has been shown that the indifference point has a utility or preference of .5, the average of the lottery. A similar procedure can be used to establish several other points along the utility function and a curve can be fitted through those points. This was repeated for each of the six indicators.

Preference curves for each indicator differed between individuals. Many participants felt that an Indian catch of 40,000, near the current level, was really sufficient and the participants' utility would not be increased greatly by increasing the Indian catch above 40,000. Graphically this can be represented by curve I in Fig. 1. This individual gains little utility by increasing Indian catch above 40,000. Curve II in Fig. 1 represents a person whose utility is increased by increasing Indian catch at any level. This does not mean that individual I thinks Indian catch is less important than individual II, it only means that individual I does not gain much utility by increasing the Indian catch above 40,000. In practice it has been found that the choice of indicators can make a substantial difference in the final utility function and the indicators should be chosen carefully.

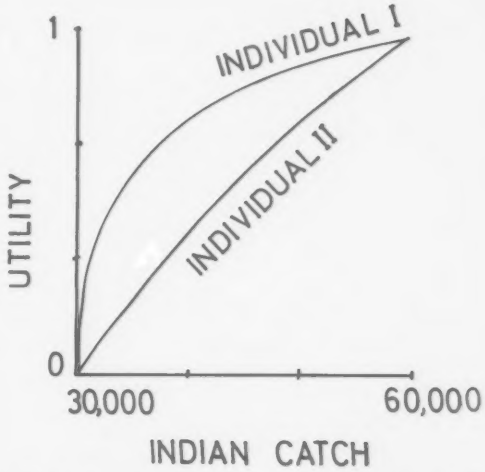

FIG. 1. Utility functions of two individuals for Indian catch. Individual I does not have his utility increased significantly by increasing Indian catch above 40,000. The utility of individual II is increased fairly uniformly over all ranges of Indian catch.

Calculations of Weightings

The second step in the utility analysis was to determine the relative importance weightings of the indicators for each individual. This step requires the assumption of "preferential independence." Preferential independence means that the tradeoffs between two indicators are independent of the values of the other indicators. For example, preferential independence requires that an individual's tradeoffs between commercial return and sports catch are independent of the current value of Indian catch. This assumption was felt to be reasonable for almost all combinations of variables. A possible exception was the fact that native Indians are members of both the commercial fishermen's group and the aboriginal Indian fishery. Someone might be more willing to give up more commercial catch to increase sports catch if the Indian catch were high than if it were low. However, it was felt that this was a small consideration in light of the large number of commercial fishermen, and the workshop participants were probably not considering this complexity when questioned about tradeoffs.

The actual questions about pair-wise tradeoffs were done as in the following example. A specific pair of indicator values was specified: 70,000 sports catch and \$20 million commercial return. Each person was then asked the following question, "If we were going to lower the recreational catch to 20,000, how much would I have to increase commercial return so that you would be indifferent to the drop in sports catch?"

First, two pairs of points that are indifferent have to be established; then we can calculate the relative values of the two indicators. We are not

TABLE 3. Weights assigned by each individual to different indicators.

Indicator/ Individual	Additional cost of management	Commercial revenue	Indian catch	Traditional sports catch	Total run size	Inputs to regional economy
I	.13	.30	.01	.43	.06	.23
II	.54	.40	.08	.29	.48	.20
III	.38	.99	.05	.02	.02	.26
IV	.11	.80	.03	.31	.15	.42
V	.44	.70	.03	.57	.32	.34
VI	.17	.50	.11	.36	.36	.38
VII	.07	.40	.09	.18	.29	.47

saying that an individual would always trade off x many of attribute A for y many of attribute B. We are trading off "utility units" of A and B. So long as the preference functions for the individual indicators are not linear, more commercial return is required to compensate for a decrease in sports catch from 21,000 to 20,000 than would be required to compensate for a decrease from 70,000 to 69,000. Participants were reluctant to accept this concept, thinking they were being asked to specify how much of A they would give up for a unit of B. They naturally responded that it depended on how much of A and B they had.

Quantitative Assessment of Enhancement Outcomes

From the information about indicator preferences and tradeoffs derived in the previous sections, the preference function for each individual was constructed. Table 3 lists the weights each individual assigned to each indicator. Please note that the sum of the weightings is not equal to 1.0. This is because the functions are "multiplicative" and one cannot compare the weightings between individuals, only the relative weights assigned to the indicators for any one individual.

From the preference functions a numerical rating between 0 and 1 was calculated for each outcome. For enhancement alternatives with more than one possible outcome, we calculated an expected preference value. These preferences are presented in Fig. 2.

Discussion

The major feature of both the intuitive and quantitative assessments was that most participants preferred alternative 4, the low-technology, all-stock enhancement. A more surprising result was the generally poor rating the no-change alternative received in the quantitative assessment. Prior to the quantitative analysis, several participants had rated this outcome the first or second most preferred, yet in the quantitative ratings it came out least preferred in all cases but one. Participant IV was the most striking. His "intuitive" preference for no change was first, yet it came out last in the quantitative ratings. Discussion of this result produced a consensus: most participants felt that there were far more risks involved in any of the three enhancement alternatives than in no-change. They also felt that the risks had been underestimated for the quantitative outcomes listed in Table 2. When the "gut feeling" assessment was done, the participants had used their assessment and aversions, rather than just the numbers in the table.

An interesting result is that all but one participant chose the same option as being "best" by both techniques. The discrepancies in techniques were revealed for the suboptimal options. It may be that people can usually decide which option they prefer most, but have trouble deciding between the lower ranked alternatives, yet frequently decision makers must choose a lower ranked option because of some constraint that eliminates his most preferred. Utility analysis may prove to be most useful in assessing preferences between less preferred alternatives.

There were wide differences in the individuals' preferences (Table 3 and Fig. 2). This was known before we began the exercise, but the workshop permitted us to make an approximation of the quantitative nature of the differences. Given that the decision maker must balance several interest groups, it seems clear that some method of getting a statement of the different interest groups' objectives should be used. To rely on a casual perception of how interest groups perceive the managed system seems foolhardy. Certainly multiattribute utility analysis is not the only possible technique, but at least it is a technique that can be used with a relatively small investment in time.

Another dominant concern that came out more in the discussion than the quantitative analysis

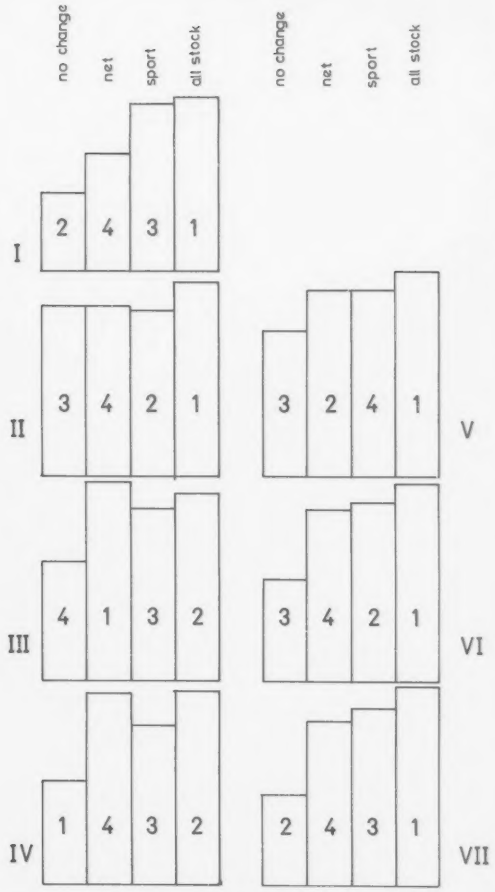

FIG. 2. Relative preferences assigned by each individual to different outcomes of enhancement policies using the derived multiattribute utility functions. Each histogram represents one individual (designated by Roman numerals). The height of the histogram bar represents the relative preference for that enhancement alternative. The numbers in the bars represent the "gut feeling" preference order.

was the difference in individuals' perceptions of risk. There are two types of risk: the first is how likely an individual thinks something will go wrong, and the second is how bad the individual perceives the problem to be when it arises. The first type is crudely expressed in the probability figures in Table 3, and the second type comes out in the slope of the utility functions such as Fig. 1. Since risk of failure is a feature of most biological and resource systems, much more time should be spent analyzing peoples' perceptions of risk. Cost–benefit analysis can handle the first type of risk, but assumes by placing a dollar value on an output, that the second type of risk is not important.

Summary

All workshop participants agreed that the experience of attempting to use the quantitative ranking technique for preference assessment was valuable. Multiattribute utility analysis appears to be a valuable format for discussion of the goals of management, providing an excellent focus for identification of points of conflict or disagreement. There was no attempt to determine an "optimal utility function" for use in management. We were simply trying to uncover individuals' preferences. Multiattribute utility analysis was successful because it forced the participants to state exactly why they preferred one outcome to another. The early stages of interest group and indicator identification were useful for identifying the groups in conflict.

There was considerable disagreement about the potential for using multiattribute utility analysis to determine interest group preferences. Several participants immediately saw opportunities in their operations to use multiattribute utility analysis to evaluate preferences. Other participants felt that the technique was a good tool to promote discussion, but should not be used with public groups due to unreliability of responses.

Recent methodological advances in systems analysis permit us to deal fairly easily with the inherent uncertainties in biological systems (Walters 1975a; Walters and Hilborn 1976). We can determine how to manage systems that have high variability in biological components. Unfortunately, our understanding of management goals and peoples' preferences lags far behind. We are only beginning to have methods for determining preferences for deterministic outcomes. We have no way of dealing with uncertainty about future objectives. As a result of this workshop and our earlier work on a wide range of problems of salmon management, we strongly recommend that considerably more effort be put into consideration of preferences and some new work be initiated on methods of dealing with uncertainties about changes in people's goals. Analysis of preferences should be an integral part of any resource management exercise.

Acknowledgments

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Encounter Probabilities and Community Structure in Zooplankton: a Mathematical Model

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GERRITSEN, J., AND J. R. STRICKLER. 1977. Encounter probabilities and community structure in zooplankton: a mathematical model. J. Fish. Res. Board Can. 34: 73–82.

Predator–prey interactions between swimming animals of the zooplankton are studied in a mathematical model. The assumptions are: 1) the animals are points in a 1-m^3 homogeneous space, 2) the animals move at random and are randomly distributed, and 3) the predator animal has an encounter radius given by its sensory system. The mathematics of encounter probabilities are developed for a 3-dimensional space. The results show two optimal strategies: 1) cruising predators which prey upon slow moving animals (herbivores), and 2) ambush (nonmoving) predators which prey upon fast cruising prey. Of the variables used (population densities, speeds of the two animal species, and encounter radius) the encounter radius has the greatest influence on the encounter probabilities. The results suggest a simple community structure and point to the importance of studies on live zooplankton.

GERRITSEN, J., AND J. R. STRICKLER. 1977. Encounter probabilities and community structure in zooplankton: a mathematical model. J. Fish. Res. Board Can. 34: 73–82.

Les auteurs étudient, à l'aide d'un modèle mathématique, les interactions prédateurs–proies entre les animaux pélagiques du zooplancton. Les hypothèses sont les suivantes : 1) les animaux sont des points dans un espace homogène de 1 m^3, 2) les animaux se déplacent au hasard et sont répartis au hasard et 3) le rayon de rencontre du prédateur est déterminé par son système sensoriel. On établit mathématiquement les probabilités de rencontre dans un espace à trois dimensions. Les résultats indiquent deux stratégies : 1) prédateurs en maraude saisissant des animaux qui se déplacent lentement (herbivores) et 2) prédateurs à l'affût (immobiles) qui se nourrissent de proies nageant rapidement. Parmi les variables utilisées (densités de populations, vitesses de deux espèces d'animaux et rayon de rencontre), le rayon de rencontre est celui qui a la plus grande influence sur les probabilités de rencontre. Les résultats suggèrent une structure de communauté simple et font ressortir l'importance d'études de zooplancton vivant.

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PREDATION influences the species composition and abundance of zooplankton in pelagic systems (Brooks and Dodson 1965; Brooks 1968; Dodson 1970, 1974; Confer 1971; Zaret 1972; Steele 1974; Kerfoot 1975). Zooplankton communities have two kinds of predators: other invertebrates and vertebrates. Vertebrate predators have the following characteristics: 1) they are visual predators that select large or otherwise easily visible prey (e.g. Ivlev 1961; Brooks and Dodson 1965; Zaret 1972; Zaret and Kerfoot 1975), 2) they are considerably larger than their planktonic prey, and 3) they swim much faster than their prey. In contrast, invertebrate predators 1) select mostly small prey due to upper size limits imposed by their grasping appendages (McQueen 1969; Dodson 1970, 1974; Swift and Fedorenko 1975; Kerfoot 1975), 2) use mechanical perception to detect their prey (Horridge and Boulton 1967; Newbury 1972; Strickler 1975a), and 3) are only slightly larger than their prey and are evolutionarily closely related to them.

As the overall effects of predation in zooplankton communities are emerging from these and other studies, and as our knowledge advances on the mechanisms of prey detection in the zooplankton (Strickler 1975a; Zaret and Strickler unpublished data), it is now possible to construct a mathematical model of predator–prey interactions in 3-dimensional space. This will allow us to

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identify the critical factors controlling predation in pelagic communities, to predict evolutionary responses of predators and prey to such interactions, and to predict the role of predation in controlling community structure. This first attempt deals with planktonic communities of low diversity, where the water is well mixed and the animals are widely dispersed, such as in boreal lakes of Canada (Davis 1975). However, due to the abstract nature of the model, it may be applied to other ecosystems.

Structure of Predation

Predation within an ecosystem can be considered to be the sum of all interactions between single predators and single potential prey animals. A single interaction can result in success or failure for the predator, corresponding to death or escape for the prey.

If we define a successful interaction as one resulting in a meal for the predator, then the rate of successful interactions is the rate of predation. For a given predator or prey, within a certain time interval, there is an associated probability of a successful interaction. To examine this, we dissect predatory interactions further.

A complete predatory interaction can be broken down into a series of chronological events, culminating in ingestion. The number of events is somewhat arbitrary: Edmunds (1974) considers two events, encounter and ingestion. For the purposes of zooplankton predation, we will consider a predatory interaction to consist of four chronological events: 1) the animals must first encounter each other, 2) the predator must recognize the other animal as a potential prey item and attack it, 3) it must capture the prey, and 4) it must successfully ingest the prey. Note that each of the animals can have a set of adaptations to change the probabilities in its favor at each of the four steps.

We define an encounter as an event when a predator and its prey are close enough so that the predator will recognize the other animal 50% of the time. If there is no encounter between predator and prey, there can be no predation. Hence, the rate of encounters will directly affect the subsequent steps, and therefore will affect the amount of food a predator eats. The importance of the encounter rate has been shown in various laboratory studies (Ivlev 1961; Holling 1965, 1966; Ambler and Frost 1974; Salt 1974), where the number of prey eaten per predator was dependent on prey density.

For the purposes of this model, we assume zooplankters to be very small animals, randomly distributed and moving in a large, homogeneous 3-dimensional environment. Within an entire lake or ocean, zooplankters are not randomly distributed (Hutchinson 1967); however, on a scale relevant to a model of predatory dynamics (about 1 m^3 and 1 h), a random distribution may be assumed.

The problem of search and encounter probabilities for randomly moving objects has been addressed by Kohlas (1967) for aircraft of the Swiss Air Force. Kohlas considered the problem of aircraft encounters to be essentially 2-dimensional, and followed the treatment developed by Koopman (1956) for naval operations research. In view of the similarity of the problem, we follow Koopman's reasoning in the development of the encounter model, with the important distinction that we develop here the 3-dimensional case of the problem.

Model

Encounter Rate

The assumptions of the model are as follows: 1) the animals are considered dimensionless points in space, 2) the animals are randomly distributed, 3) the animals are swimming in random directions, with a uniform probability distribution, 4) the mean swimming speed of each species will be used in the derivation, and 5) predators have an encounter radius, R, which is assumed constant in all directions.

A logical frame of reference is spherical polar coordinates, with the predator at the origin (Fig. 1). Consider an element of solid angle, $d\omega$:

$$d\omega = \sin\theta d\theta d\phi$$

If a single prey swims towards the origin with an unknown track angle, Ω, relative to the predator, where the track angle Ω is defined as the spherical coordinates θ and ϕ of the path of the prey, and Ω has a uniform probability over its range (assumption 3), then the probability that Ω is in the element $d\omega$ is proportional to $d\omega$:

$$P(\Omega)d\omega = kd\omega = k\sin\theta d\theta d\phi$$

Ω exists; therefore, the probability that Ω occurs within some $d\omega$ is 1:

$$1 = \int P(\Omega)d\omega = \int_0^{2\pi}\int_0^{\pi} k\sin\theta d\theta d\phi = 4\pi k$$

Therefore, $k = 1/4\pi$ and

$$P(\Omega)d\omega = (1/4\pi)\sin\theta d\theta d\phi \qquad (1)$$

If there are N_H uniformly distributed prey per

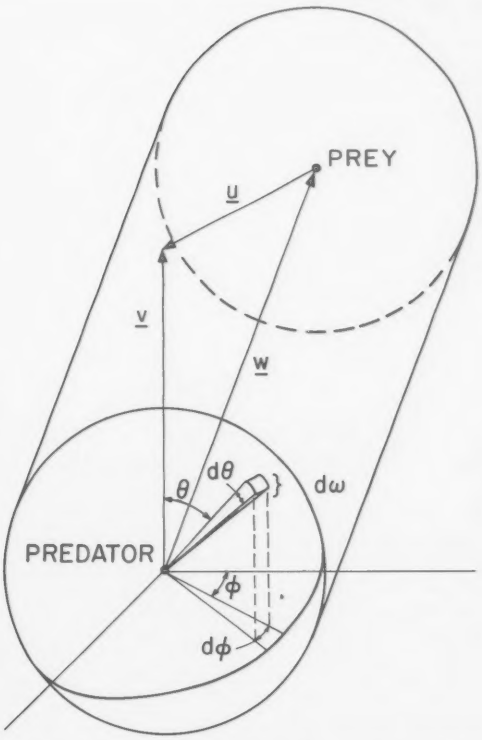

FIG. 1. The encounter sphere of a predator and the volume it sweeps as the predator searches for prey. See text for explanation.

unit volume, the expected number with a track angle in $d\omega$ is

$$(N_H/4\pi)\sin\theta d\theta d\phi$$

The subscript H refers to prey; the subscript P refers to predators. Thus, N_H is the density of prey, and N_P is the density of predators.

Consider a predator with an encounter radius R, swimming with velocity $\boldsymbol{v}$ through the water, and a prey animal swimming with velocity $\boldsymbol{u}$. The magnitudes of the velocity vectors $\boldsymbol{v}$ and $\boldsymbol{u}$ are the speeds v and u. The z-axis ($\theta = 0$) of the coordinate system lies along vector $\boldsymbol{v}$. The track angle of the prey is the spherical coordinates θ and ϕ of the vector $\boldsymbol{u}$ with respect to $\boldsymbol{v}$. A prey has a relative velocity $\boldsymbol{w}$ with respect to the predator:

$$\boldsymbol{w} = \boldsymbol{v} - \boldsymbol{u} \tag{2}$$

and the relative speed w is given by the law of cosines:

$$w = \sqrt{u^2 + v^2 - 2uv\cos\theta} \tag{3}$$

To enter the encounter sphere during a time interval t, a prey animal, at time t_0, must be in the cylindrical volume that the encounter sphere sweeps with relative velocity $\boldsymbol{w}$ during the interval t. This volume has height wt and base area πR^2. The number of prey, with a track angle in $d\omega$, entering the sphere per unit time is $\pi R^2 w (N_H/4\pi)\sin\theta\, d\theta\, d\phi$. Integrating over θ and ϕ gives Z_P, the encounter rate of a predator with its prey, or the total number of prey entering the sphere per unit time:

$$Z_P = \frac{\pi R^2 N_H}{4\pi}\int_0^{2\pi}\int_0^{\pi} w\sin\theta d\theta d\phi$$

$$Z_P = \frac{R^2 N_H}{4}\int_0^{2\pi}\int_0^{\pi} \times \sqrt{\bar{u}^2 + v^2 - 2\bar{u}v\cos\theta}\sin\theta d\theta d\phi \tag{4}$$

where $\bar{u}$ is now the mean speed of the prey population, rather than the speed of an individual prey animal.

Evaluating for ϕ:

$$Z_P = \frac{\pi R^2 N_H}{2}\int_0^{\pi}\sqrt{\bar{u}^2 + v^2 - 2\bar{u}v\cos\theta}\sin\theta d\theta$$

and for θ gives:

$$Z_P = \frac{\pi R^2 N_H}{6}\frac{(\bar{u} + v)^3 - |\bar{u} - v|^3}{\bar{u}v} \tag{5}$$

Equation (5) reduces to:

$$Z_P = \begin{cases} \dfrac{\pi R^2 N_H}{3}\left(\dfrac{\bar{u}^2 + 3v^2}{v}\right) & \text{for } v \geq \bar{u} \quad (6) \\ \dfrac{\pi R^2 N_H}{3}\left(\dfrac{v^2 + 3\bar{u}^2}{\bar{u}}\right) & \text{for } \bar{u} \geq v \quad (7) \end{cases}$$

Note that (6) and (7) also express Z_H, the rate of encounter of a prey animal with its predator population, if N_H is replaced by N_P, the density of predators, and if v is replaced by $\bar{v}$. The total rate of encounter between predators and prey per unit volume, Q, is given by multiplying the rate of encounter for a single predator by the density of predators:

$$Q = Z_P N_P \tag{8}$$

We have assumed that using the mean speed of a population in the model gives the same results as if all animals in the population were swimming at exactly the same speed. More precisely, equation (5) must be integrated over u:

$$Z_P = \frac{\pi R^2 N_H}{6}\int_0^{\infty} \times \frac{(u + v)^3 - |u - v|^3}{uv} f(u)du \tag{9}$$

where $f(u)$ is the probability distribution of u. This equation is best solved numerically for the individual distribution of u, because of the absolute value in the integral. Similarly, for Q, equation (9) must be integrated over v:

$$Q = \frac{\pi R^2 N_H N_P}{6} \int_0^\infty \int_0^\infty \times \frac{(u+v)^3 - |u-v|^3}{uv} f(u)g(v)dudv \quad (10)$$

where $g(v)$ is the probability distribution of v. For the present we will use the mean speeds in the model, and thus equations (6) and (7) express the basic encounter rate model.

Encounter Probability

Randomly occurring, independent events over time are Poisson distributed (Fry 1965; Kohlas 1967). The Poisson parameter λ is the rate of encounter multiplied by the time interval under consideration:

$$\lambda_H = Z_H t, \quad \lambda_P = Z_P t$$

The probability of a predator's encountering x prey animals during time interval t is then:

$$P(x, t) = e^{-Z_P t} \frac{(Z_P t)^x}{x!} \quad (11)$$

The probability of a prey animal's encountering x predators is given by replacing Z_I, with Z_{II} with equation (11).

Encounter Radius

As most zooplankters depend on mechanical signals for information about nearby animals, the encounter radius, R, is a complex function of many variables and will not be constant under all circumstances. Whether or not an animal detects a disturbance in the water depends on the strength of the disturbance, the distance between the disturbance and the animal, the sensitivity of the animals' receptors, and the relative level of noise (such as turbulence) in the system. An animal that is moving, moreover, creates pressure and shear disturbances in front, in back, and alongside of it (Strickler 1975a, b; Zaret and Strickler unpublished data). These disturbances may combine to reduce the sensitivity of the animal's receptors and to increase the level of noise; moreover, the disturbances should increase with increasing speed, resulting in a reduction of the potential detection radius as a predator swims faster.

To see how the above effects might change the behavior of the model, appropriate relations were incorporated into a simulation of the model. As a first approximation, the following assumptions were made concerning R: 1) the size and speed of the prey have no effect on R, and 2) the predator's encounter radius, R, decreases exponentially with the predator's speed to a certain minimum, which corresponds to direct contact between the two animals. The following two relations were used:

$$R = c_1 e^{-c_2 v} + c_3 \quad (12)$$

$$R = c_1 e^{-c_2 v^2} + c_3 \quad (13)$$

where c_1 and c_2 are proportionality constants and c_3 is the minimum, direct-contact radius.

Energetic Efficiency

For an animal to grow and reproduce, the energy that it obtains from its food must be more than the energy spent to acquire the food. The relative amount of energy available for growth and reproduction may be expressed as the animal's efficiency, W, defined as the ratio of maximum expected energy gain per unit time, or power input, P_i, to energy spent per unit time, or power output, P_o:

$$W = \frac{P_i}{P_o} \quad (14)$$

Seasonal energy storage was ignored to arrive at an animal's average efficiency over its life span.

Power input, P_i, or food eaten per unit time, was defined as a constant proportion of the encounter rate:

$$P_i = \alpha Z_P \quad (15)$$

where α represents the proportion of prey encountered that are actually ingested, times the energetic value of the prey. The constant α expresses the probability of attack, capture, and ingestion, given an encounter, but for the purpose of this model it does not take into account functional responses of predators (Holling 1966).

The power output, P_o, was defined as the energy spent swimming plus a constant metabolic rate. The drag force on swimming animals of the same size and shape is proportional to the square of the speed (Shapiro 1961; Vlymen 1970), and the power required to swim is thus proportional to the speed cubed (Klyashtorin and Yarzhombek 1973):

$$P_o = \beta v^3 + \gamma \quad (16)$$

TABLE 1. Percent deviation of encounter rates Z_P and Q calculated with mean speeds $\bar{u}$ and $\bar{v}$ (equations 6, 7, 8) from the correct values of Z_P and Q obtained with numerical integration (equations 9 and 10). Right-hand columns give percent deviation. Experimental speed distributions were generated by a pseudorandom number generator.

Case	Predator speed distribution	$\bar{v}$	Prey speed distribution	$\bar{u}$	% deviation Z_P	% deviation Q
$\bar{v} > \bar{u}$	uniform	0.40	uniform	0.10	−0.7	−0.6
	uniform	0.40	normal	0.10	−0.6	−0.5
	normal	0.41	normal	0.10	−0.5	−1.1
	normal	0.41	log-normal	0.11	−1.7	−2.4
	log-normal	0.39	log-normal	0.11	−1.9	−6.5
	log-normal	0.39	uniform	0.10	−0.7	−5.0
$\bar{v} \simeq \bar{u}$	uniform	0.39	uniform	0.40	−0.5	−0.8
	uniform	0.39	normal	0.39	−4.0	−4.2
	normal	0.41	normal	0.39	−3.8	−7.1
	normal	0.41	log-normal	0.38	−10.7	−12.7
	log-normal	0.42	log-normal	0.38	−10.7	−16.9
	log-normal	0.42	uniform	0.41	−0.5	−12.2
$\bar{v} < \bar{u}$	uniform	0.10	uniform	0.41	−0.04	−0.6
	uniform	0.10	normal	0.36	−1.8	−2.7
	normal	0.11	normal	0.36	−2.0	−2.5
	normal	0.11	log-normal	0.41	−3.8	−4.3
	log-normal	0.10	log-normal	0.41	−3.7	−5.5
	log-normal	0.10	uniform	0.40	−0.05	−1.9

The constant γ expresses all elements of total metabolism (Kerr 1971a, b) not contributing to growth, reproduction, or capture of prey; β is a constant approximating other variables of the drag function. The proportion of a zooplankter's energy spent swimming is unclear; estimates of energy spent in diurnal vertical migrations range from very low (Hutchinson 1967; Vlymen 1970) to as high as 40% (Klyashtorin and Yarzhombek 1973) or 95% of metabolic rates (Petipa 1966).

Results and Predictions

Effect of Speed Distributions

Mean swimming speeds were used in the calculations of encounter rates (equations 6, 7, 8), although real zooplankters do not swim at a constant speed. Encounter rates obtained from mean speeds were compared with encounter rates obtained by numerical integrations of equations (9) and (10) (Table 1). Three distribution functions for speeds ($f(u)$ and $g(v)$, equations 9 and 10) were used: uniform, normal and log-normal, and three cases of the relative values of the mean speeds: $\bar{v} > \bar{u}$, $\bar{v} \simeq \bar{u}$, and $\bar{v} < \bar{u}$. Calculations based on mean speeds consistently underestimate encounter rates; the magnitudes of the errors are determined by the speed distributions and by the relative values of the means. Therefore, mean speeds may be used to calculate adequate approximations of encounter rates in most cases. Correction factors are required only for log-normally distributed speeds.

Effect of Speed

The encounter rate for predators or prey, Z (equations 6 and 7), is an increasing function of four independent variables: N_{II} or N_I, R, v, and u. The four independent variables are by definition positive and the first partial derivatives of Z are positive; hence, Z is an increasing function. This indicates that predators may increase their encounter rate with prey by increasing their encounter radius or their speed, and, conversely, that prey may decrease their encounter rate by decreasing their speed or the predator's encounter radius.

If predators and prey have different mean speeds, the greater of the two speeds has the greatest effect on the encounter rate (Fig. 2 and 3). This effect is also apparent from equations (6) and (7) and from the partial derivatives of Z with respect to u and v. When the speed of a prey animal is less than that of its predator, a further decrease in speed does not give as great a decrease in encounter rate or encounter probability. If an animal swims very slowly, it will also have a lower rate of encounter with its own food and with potential mates; therefore, a prey animal is expected to have a slower cruising speed

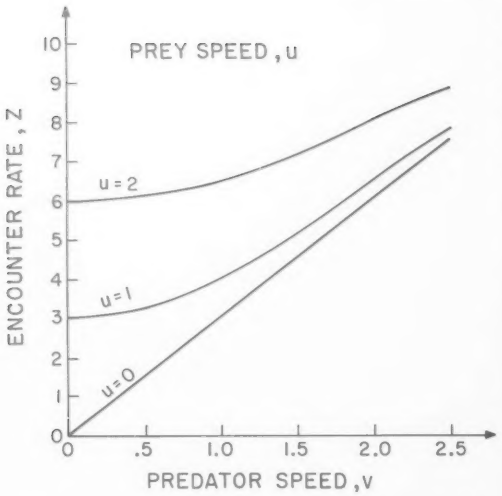

FIG. 2. The encounter rate, Z, as a function of predator speed, v, for three different prey speeds, u. $R = 1$, $N_{II} = 1$. Note that when $u = 0$, the encounter rate is linear with v, and when $u > 0$, the encounter rate approaches this line asymptotically. All variables are nondimensional to show the behavior of the model (the same in Fig. 2–6).

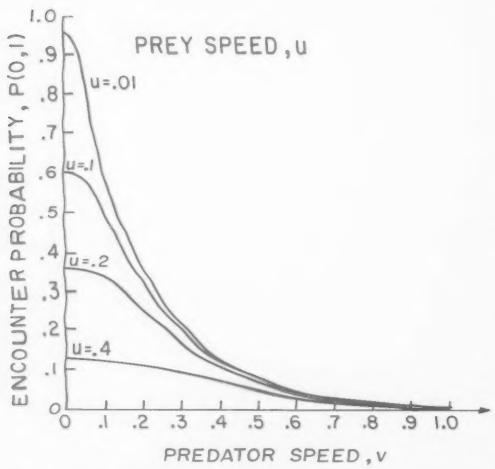

FIG. 3. Poisson probabilities of no encounter [P (0, 1) equation 11, $x = 0$, $t = 1$] as a function of predator speed, v, for different prey speeds, u. Encounter radius $R = 0.3$, prey density $N_{II} = 5$. Optimal probabilities for predators are lower, and optimal probabilities for prey are higher. Note the sharp decline in the probability of no encounter when prey speeds are low, and the relatively slower decline when prey speeds are higher.

than its major swimming predator, but this speed should not be zero. Conversely, if a predator is slower than its prey, an increase in the predator's speed gives only a slight increase in the encounter rate. Substantial increases in encounter rate will occur only if the predator's speed is as great or greater than that of the prey.

Effect of Encounter Radius

The highest-order variable in equations (6) and (7) is the encounter radius, R; hence, an increase in R should produce the greatest relative increase in the encounter rate and encounter probability (Fig. 4). A slight increase in R results in a considerable decrease in the probability of no encounter (Fig. 4A), while an increase in v has a smaller effect (Fig. 4B).

When equation (13) is included in the model, there are two predator speeds that result in maximum encounter rates, one at low prey speeds, the other at higher prey speeds (Fig. 5). This relation is sensitive to the values of the constants c_1, c_2, and c_3 (equation 13); if c_1 is small or if c_3 is large, the encounter rate increases with increasing v. Equation (12) gives a similar figure, but it is much more sensitive to the values of the constants. These results emphasize the close relationship between the performance of the sensory system and the ability of a predator to capture prey.

Efficiency

Within the framework of the encounter model, we would like to know the variables, for a predator, giving maximum efficiency in the utilization of a food resource. We assume that over evolutionary time, natural selection perpetuates those predators that catch the most food relative to their swimming power output. The efficiency includes the effect of increasing drag force as an animal increases its cruising speed (equations 14, 15, and 16). Two types of predators have optimal efficiency under these conditions (Fig. 6): stationary, or ambush, predators are most efficient at utilizing fast prey (curve of $u = 2.0$, Fig. 6), and cruising predators are most efficient at utilizing slow prey (curve of $u = 0$, Fig. 6). As an animal swims faster, its drag force increases, and it must expend more energy to overcome drag. Consequently, a faster animal encounters more prey (Fig. 2 and 3), but it uses more energy. To be energetically efficient, it must catch prey in proportion to the exponentially increasing energy expenditure. The effect becomes strong at high speeds (Fig. 6, right-hand side), where the energy

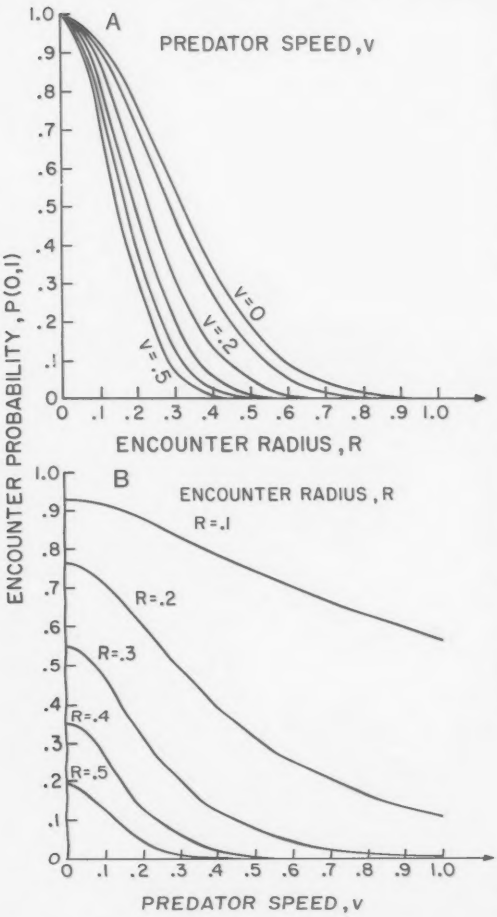

FIG. 4. Poisson probability of no encounter [P (0, 1), equation 11, $x = 0$, $t = 1$], A, (upper graph) as a function of encounter radius R for different predator speeds v, B, as a function of predator speed v for different encounter radii, R. Prey speed $u = 0.1$, prey density $N_H = 5$. A and B are orthogonal views of the same 3-dimensional surface.

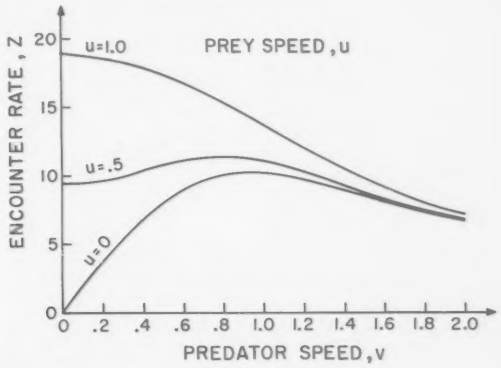

FIG. 5. Encounter rate, Z, as a function of predator speed, v, for different prey speeds u, when encounter radius, R, declines with increasing predator speed (equation 13). $N_H = 0.67$.

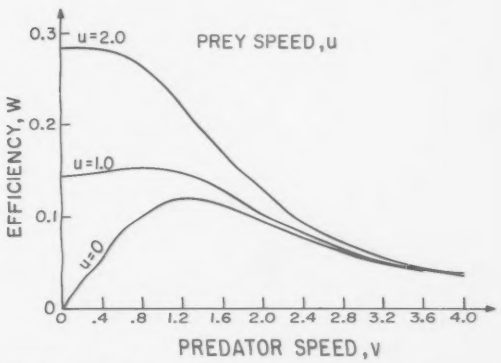

FIG. 6. Predator efficiency, W, as a function of predator speed v, for different prey speeds, u. Efficiency, W, is power input ($P_i = \alpha Z_P$, equation 15) divided by power output ($P_o = \beta v^3 + \gamma$, equation 16). Prey density $N_H = 1$, $\alpha = 1$, $\beta = 0.5$, $\gamma = 2$.

intake is no longer proportional to the energy expenditure, and as a result efficiency declines. If prey are fast moving, efficiency declines immediately, and an ambush predator is most efficient. In the case of very slow moving prey, the encounter rate increases faster than energy expenditure as a predator swims faster, up to an optimum, moderate speed.

The constants α, β, and γ have no effect on the basic shapes of the curves (Fig. 6), but they do affect the scale of the curves. β has the most pronounced effect: a decline in β ($\beta \to 0$), equivalent to better swimming efficiency, results in a higher efficiency, W, and a faster optimal cruising speed, v, on the curve $u = 0$. An increase in γ also shifts the maximum on the curve $u = 0$ to the right, but lowers the value of W.

Two other results are of note. 1) The curve for $u = 1.0$ (Fig. 6) has a shoulder as a maximum from $v = 0$ to $v = 1.2$. Any predator speed in this range works with nearly the same efficiency. 2) Figure 6 shows similar results to Fig. 5, where we linked the encounter radius to the speed of the predator. This similarity clearly shows that there are two reasons for the existence of two optimal

predator cruising speeds: 1) higher energetic efficiency, and 2) a better relationship between signals per unit time and the general noise level. This holds for all speeds of the prey.

Discussion

Strategies[2] and Community Structure

The model clearly predicts two optimal cruising strategies for predators: ambush predation and cruising predation (Fig. 5 and 6), each most efficient at utilizing a certain prey resource. Our model predicts that, in a classic situation of food competition between two predators, each using a different strategy, the swimming behavior of the prey will determine the outcome. Both predators have a higher encounter rate and a greater efficiency with fast prey than with slow prey. However, the ambush predator wins against the cruising predator if the prey is fast moving.

The existence of two discrete types of predator strategies and their specialization on prey with different speeds suggests a mechanism for resource partitioning (MacArthur 1972; Schoener 1974) between the two predator types. However, resource partitioning depends on competitive displacement according to the Gause-Volterra principle (Hutchinson 1958), and the encounter model does not consider (as an input) competition between predators. The model and its predictions are based mainly on physics and probability; thus, the two strategies predicted are not the result of competition. An ambush predator is, in effect, an obligate specialist on fast-moving prey, and will not compete with a predator that uses only slow prey. Cruising predators can be generalists on prey of all speeds, and could switch strategies (Murdoch 1969), thus increasing the probability of competition.

For prey, the best strategy to avoid encounters with predators is to move slowly (Fig. 2 and 3). However, prey animals must also encounter mates and their own food; filter feeders must filter new water, and omnivores must swim to encounter food particles. Very slow animals have a very low probability of encountering ambush predators and a moderate probability of encountering cruising predators. Hence, slow prey need secondary defenses (Edmunds 1974) only for cruising predators. Faster prey animals, however, have high probabilities of encountering ambush and cruising predators and need secondary defenses for both types of predators.

Considering the strategies and observations above, we may now outline a simple, logical community structure based on speed and trophic level. The primary producers, or phytoplankton in pelagic environments, move passively or very slowly, enough to encounter fresh nutrients (Titman and Kilham 1976). Herbivores grazing on them need some minimum motion to encounter the phytoplankton, but also must swim slowly to avoid encountering their own predators. Filtering currents of planktonic herbivores may serve to increase an animal's encounter rate with phytoplankton. Newton's third law of motion does not allow the animal to remain stationary when it is filtering (special swimming modes may have evolved to counteract this). The slow-swimming herbivores are subject to predation by cruising predators, the first carnivores. The presence of faster-swimming animals now allows the existence of secondary ambush predators, which cannot exist on the slow-swimming herbivores alone. This simple primary structure will increase in complexity as prey species show escape reactions to avoid being captured and predators find alternative food with different behavioral repertoires.

Central to our hypothesized community structure is the relationship between cruising speed and efficiency. We have not assigned units to the speeds in our simulations, as we are only interested in the general behavior of the model. The predator and prey cruising speeds in Fig. 6 are in the same, nondimensional units. The value of the optimal speed of a cruising predator will be determined by the values of the constants β and γ in the expression of power output (equation 16). Swimming efficiency, approximated by β, is a complex function involving hydrodynamic efficiency, propulsion efficiency, and muscular efficiency. A decrease in β (i.e. greater swimming efficiency) results in an increase in the optimum speed for cruising predators. In other words, more efficient swimmers will have a higher optimal cruising speed as cruising predators. Fish are more efficient swimmers than zooplankton (Schmidt-Nielsen 1972); therefore, planktivorous fish will have a higher encounter rate than zooplankton.

There are abundant examples in the literature of ambush and cruising predators. Some examples of ambush predators are chaetognaths (Horridge and Boulton 1967), corals preying on passing zooplankton (Porter 1974), and midge larvae preying on herbivorous and predatory zooplankters (Swift and Fedorenko 1975). Cruising predators are also found; many predaceous copepods,

[2]We recognize that the word "strategy" is anthropomorphic, and we use it here merely to facilitate communication. What is implied is that natural selection has perpetuated species which interact in their ecological theater in a nearly optimal manner (with so-called "strategies") to maximize their fitness (Hutchinson 1965).

such as *Cyclops* sp. and *Epischura* sp., cruise much faster than similar-sized herbivorous zooplankters (Gerritsen unpublished data). The best example is the specialization on prey by ambush and cruising predators, described by Anderson (1974), in which two ambush predators, a floating ctenophore species and a chaetognath species, fed primarily on actively swimming copepods, while a cruising ctenophore species fed on slowly drifting copepods.

Stability and Limitations of the Model

It is necessary to analyze the stability and the applicability of the model as the variables and constants change. The model is most sensitive to changes in the encounter radius (Fig. 4 and 5). The values of the constants c_1, c_2, and c_3 (equations 12 and 13), greatly influence the behavior of the model when it incorporates the assumption of reduced encounter radius with increasing predator speed (Fig. 5). The size of the encounter radius is actually a complex interaction involving predator speed, prey speed, and prey size (Zaret and Strickler unpublished data) and the encounter radius varies considerably with these three variables. This variability of the encounter radius will influence strategies available to predator and prey. Therefore, the behavior of predators' encounter radii under different conditions must first be known before more accurate modifications of the encounter model can be made.

Two assumptions central to the derivation of the model are those of random direction of motion and random distribution of planktonic animals within space. The assumptions seem reasonable within the limits, though they are slightly biased by the behavior of different zooplankters to sink rather than swim down (Strickler 1970). This fact indicates the need for further investigation.

The behavior of the predator–prey system will change when there is a high probability of more than one prey simultaneously in the encounter field of a predator. In this case, the predator has a choice of prey to attack, and the probabilities of attacking each prey are no longer constant, nor are they independent of each other. Note that this does not change the encounter rate, nor the encounter probabilities, but it does change the probabilities of attack. For simultaneous choice to occur either a predator must have a large encounter radius, or the density of prey must be high. If a large invertebrate predator 1 cm long has an encounter radius of 2 cm, its encounter sphere has a volume of about 30 ml. For there to be a high probability of more than one prey in the sphere, the density of prey must be more than 33 animals/l. This is a high figure, but is within the range observed for freshwater zooplankton (Davis 1975). Smaller predators, such as predatory copepods, have a much smaller encounter radius of about 0.5 cm (Gerritsen, in prep.), requiring an exceedingly high prey density of over 2000 animals/l for a similar effect. We see, then, that as encounter radius increases, the opportunity for choice increases. This is, in fact, the case for planktivorous fish which have a large encounter radius because they are visual predators, and when presented with a choice, they should select the most appealing (e.g. largest) prey (O'Brien et al. 1977).

The encounter model and its predictions are characteristic of any 3-dimensional predator–prey system that satisfies the constraints and assumptions of the model. The model is not derived from characteristics of planktonic animals, but rather from inherent characteristics of predator–prey interactions in 3-dimensional fluid environments. Essential to the model is a homogeneous environment, such that the probabilities of attack, capture, and ingestion remain constant over space at a given time. These probabilities must be constant regardless of where the interaction takes place (within the 1 m^3 for zooplankton). Therefore, neither predator nor prey can have a refuge or hiding place determined by the structure of the environment. Without additional assumptions the model as developed here would not apply to terrestrial or benthic ecosystems where prey have cover (e.g. foliage or rocks). In addition to most pelagic predator–prey systems, the model would apply to some aerial interactions, notably bats and birds preying on insects on the wing.

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DNA, RNA, Protein, and Free Amino Acids During Ontogenesis of Rainbow Trout (*Salmo gairdneri*)[1]

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ZEITOUN, I. H., D. E. ULLREY, W. G. BERGEN, AND W. T. MAGEE. 1977. DNA, RNA, protein, and free amino acids during ontogenesis of rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 34: 83–88.

During the first 2 wk of incubation, the pattern of change in DNA concentration tended to precede similar changes in RNA concentration. Unfertilized eggs contained 23.2 and 77.2 μg of DNA and RNA, respectively. DNA concentration rose from 155.8 μg/fry (after 23 days of incubation) to 326.6 μg/larva (14 days after hatching), while RNA increased from 238.8 to 693.3 μg during the same period. Protein per ontogenetic unit was relatively constant during the prehatching stages but dropped markedly just prior to hatching. Of the protein in the unfertilized egg, approximately 20% was retained in the fry at hatching and approximately 50% was retained in the larva following yolk absorption. Total free amino acid concentrations declined just after fertilization, indicating the initiation of protein synthesis or a decline in yolk protein degradation. These concentrations increased to near initial values in the blastula and were nearly doubled in the yolk plus fry at hatching.

ZEITOUN, I. H., D. E. ULLREY, W. G. BERGEN, AND W. T. MAGEE. 1977. DNA, RNA, protein, and free amino acids during ontogenesis of rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 34: 83–88.

Durant les 2 premières semaines d'incubation, les changements dans la teneur en ADN tendent à précéder des changements identiques dans la teneur en ARN. Les œufs non fécondés contiennent 23.2 et 77.2 μg d'ADN et d'ARN respectivement. La teneur en ADN s'élève de 155.8 μg/fretin (après 23 jours d'incubation) à 326.6 μg/larve (14 jours après éclosion), alors que l'ARN augmente de 238.8 à 693.3 μg durant la même période. Les protéines par unité ontogénique sont relativement constantes durant les stades d'avant-éclosion, mais diminuent notablement juste avant l'éclosion. Des protéines présentes dans l'œuf non fécondé, environ 20% sont retenues dans le fretin au moment de l'éclosion et environ 50% sont retenues dans la larve après résorption du vitellus. Les concentrations d'acides aminés libres totaux diminuent juste après la fécondation, indiquant le début de la synthèse des protéines ou une diminution de la dégradation des protéines du vitellus. Ces concentrations augmentent presque jusqu'aux valeurs initiales dans la blastule et atteignent presque le double dans le vitellus plus fretin à l'éclosion.

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GROWTH of fish embryos is dependent upon the quantity of nutrients stored in the yolk (particularly protein and energy) since the egg membrane is impermeable to amino acids and nucleotides (Neyfakh and Abramova 1974). Successful ontogenesis results from the conversion of yolk materials to the tissues of the embryo. In fish, presumably protein synthesis or the translocation of yolk protein to the blastoderm during the early stages of development is the ultimate feature of growth as it is in higher animals (Lewis and D'Mello 1968). Protein synthesis is governed by tissue DNA and RNA concentration. Enesco and Leblond (1962) and Goss (1966) described growth as an aggregate of increases in cell number (hyperplasia) and cell size (hypertrophy). Cell number can be estimated from tissue DNA concentration, while RNA concentration is an estimate of hypertrophy. Total protein concentration of brown trout (*Salmo trutta*) eggs decreases as the days of incubation increase, while fat concentration remains more or less constant (Phillips and Dumas 1959). Suyama (1958) stated that the amino acid composition of rainbow trout (*S. gairdneri*) unfertilized eggs is similar to that of chicken yolk.

The present study was initiated to determine the total DNA, RNA, protein, and free amino acids in various ontogenetic stages and in larvae of rainbow trout (*S. gairdneri*) in order to define

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the embryological pattern of growth and the biochemical changes that take place during ontogenesis.

Materials and Methods

Eggs and Incubation

Eggs were obtained from rainbow trout (*S. gairdneri*) caught at the Little Manistee River Weir, Manistee County, Michigan. Three groups of eggs were collected, each originating from one female. A sample of each group was frozen on dry ice, and the remaining eggs in each group were fertilized by the milt of two males. One-half hour later, a second sample from each group was frozen on dry ice.

The remaining fertilized eggs were incubated in a Heath Fish Incubation Cabinet Model 1B-16 (Heath Tecna Corp., Kent, Washington), furnished with a flowing-water regulator. Filtered well water was used with a temperature of 10–16°C.

Additional samples were taken from each group at daily intervals for the first 5 days, followed by one sample every 2 days through the 2nd wk. During the remainder of incubation one sample was taken weekly. After hatching, the larvae were kept in the incubator until the yolk was completely absorbed. At this time, the final sample was obtained. (Most eggs hatched on day 23 of incubation, and complete absorption of the yolk took place 2 wk later.) All samples were stored at −20°C until analyzed. The sequence of stages sampled and their identification are listed in Table 1.

Table 1. Stage identification and number, and days of incubation of rainbow trout (*Salmo gairdneri*).

Stage no.	Days of incubation	Stage identification
1	0	Unfertilized
2	½ hr	Postfertilized (hardened)
3	1	Early clevage
4	1½	" "
5	3	" "
6	4	Early blastula
7	5	Late blastula
8	13	Eyed stage
9	17	Organogenesis
10	21	"
11	23	Hatched (yolk + fry)
12	23	Fry only
13	37	Larva (postyolk absorption)

Analytical Procedures

The number of eggs per sample was counted, and dry matter concentrations were determined on each sample by drying a portion in a vacuum of 740 mm Hg residual pressure at 65°C for 18 h.

Prior to nucleic acid and protein analysis, the eggs were thawed at 4°C and homogenized with cold, deionized distilled water (1/5, wt/vol) using a Polytron (Brinkmann Instruments Inc., Westbury, New York) at low speed. Newly hatched fry were homogenized as described after isolation and removal of the yolk.

A modification of the Munro and Fleck (1966) method was used to measure nucleic acid concentrations. Approximately 2–3 ml of the homogenate was pipetted into a 15-ml glass centrifuge tube and 5 ml of cold 5% (wt/vol) perchloric acid (PCA) was added. Tube contents were mixed with a Vortex Mixer (Scientific Industries Inc., Springfield, Massachusetts), placed in ice for 15–20 min, and centrifuged at 39,000 *g* for 15 min at 4°C. The supernatant was discarded and the precipitate dispersed in 5 ml of cold 1% (wt/vol) PCA. Tube contents were mixed, centrifuged at 39,000 *g* for 15 min at 4°C, and the supernatant discarded. The precipitate was dispersed and digested in 4 ml of 1.0 N KOH for 1–2 h at 37–40°C. During digestion the tubes were covered with marbles to limit water loss and contamination. After digestion the tubes were cooled to 4°C for 15 min. The contents were then acidified by addition of cold 5% PCA, mixed, and centrifuged for 15 min at 39,000 *g*. The supernatant was collected in 20-ml graduated tubes. The precipitate was washed twice with cold 5% PCA, mixed, and centrifuged as previously described. The two washings were added to the original supernatant and the total volume brought to 20 ml with cold 5% PCA. This supernatant contained the extracted RNA, which was quantified by the orcinol reaction for pentose (Brown 1946).

DNA was extracted from the above precipitate by dispersion in 4 ml of 10% (wt/vol) PCA. The tubes were covered with marbles and heated in a water bath at 70°C for 25 min. The contents were mixed gently 3 or 4 times during heating. After cooling, the tubes were centrifuged at 39,000 *g* for 15 min at 4°C. The supernatant was collected in a graduated tube and the precipitate washed with 3.8 ml cold 10% PCA, mixed, and centrifuged as before. The supernatant was added to the first collection and the total volume brought to 8 ml with cold 10% PCA. This supernatant contained the extracted DNA, which was quantified by the diphenylamine method of Giles and Myers (1965).

Total protein and free amino acids were determined on 2 ml of the original homogenate which was pipetted into a glass centrifuge tube and to which 5 ml of 30% (wt/vol) sulfosalicylic acid was added. The contents were mixed and the tubes placed in ice for 30–60 min. After remixing, the tubes were centrifuged at 39,000 *g* for 15 min at 4°C. The supernatant was collected, evaporated to near dryness, and 2 ml of pH 2.0 lithium citrate buffer added. This supernatant was analyzed for free amino acids on an Autotechnicon Amino Acid Analyzer (Technicon Instruments Corp., Ardsley, New York) as described by Bergen et al. (1973). The precipitate was digested in 5 ml of 1.0 N KOH at 37–40°C for 2 h. The total protein concentration of the digest was determined by Miller's (1959) modification of the Lowry et al. (1951) method.

Data were analyzed for significance using the analysis of variance. Duncan's test (Steel and Torrie 1960) was used to compare means in different onto-

TABLE 2. Dry matter, DNA, RNA, and protein concentration per ontogenetic unit (OU), and losses and retentions of protein at hatching and complete absorption of the yolk of rainbow trout (*S. gairdneri*).

Stage identification	Stage no.	Wt (*mg*)	Dry matter (%)	DNA (*μg/OU*)	RNA (*μg/OU*)	Protein (*mg/OU*)
Unfertilized	1	102.0[a]	41.3[a]	23.6[a]	77.2[a]	23.2[a]
Hatched (fry + yolk)	11	103.7[a]	30.0[b]	—	—	18.7[b]
Fry only	12	25.7[b]	32.9[c]	155.8[b]	238.8[b]	4.8[c]
Larva (postyolk absorption)	13	139.0[c]	18.0[d]	326.6[c]	693.3[c]	11.8[d]
±SE ($n = 3$)	—	6.2	1.1	14.2	16.7	1.5
Loss						
during prehatching stages, (%)[e]						19.4
from unfertilized eggs to larval stage, (%)[e]						49.1
from the yolk posthatching (%)[f]						50.0
Retention						
in fry (%)[g]						20.7
in larva (%)[g]						50.9

[a,b,c,d]Means with different superscripts under the same heading are significantly different ($P < 0.05$).

[e] $\frac{X_0 - (X_1 \text{ or } X_2)}{X_0} \times 100$, where X_1 and X_2 are the concentrations of nutrient per ontogenetic unit at hatched and larval stages, respectively, and X_0 is that of the unfertilized oocyte.

[f] $\frac{X_2 - X_3}{X_1 - X_3} \times 100$; X_3 is the nutrient concentration per fry.

[g] $\frac{(X_2 \text{ or } X_3)}{X_0} \times 100$.

genetic stages. Because the ontogenetic stages were sequential, a bias was introduced into Duncan's test, but this test still gives a useful approximation of the significance of differences among the means.

Results

Changes in DNA concentration tended to precede changes in RNA concentration by one sampling period, this relationship being particularly obvious during the first 13 days of development (Fig. 1). Following the 3rd and 5th day of incubation, DNA and RNA per ontogenetic unit gradually increased until hatching. All results reported in the present study for prehatching stages were derived from eggs without separating the yolk. DNA in the yolk-free fry (hatched stage) rose from 155.8 to 326.6 μg at the postyolk absorption (larval) stage, while RNA increased from 238.8 to 693.3 μg during the same period (Table 2).

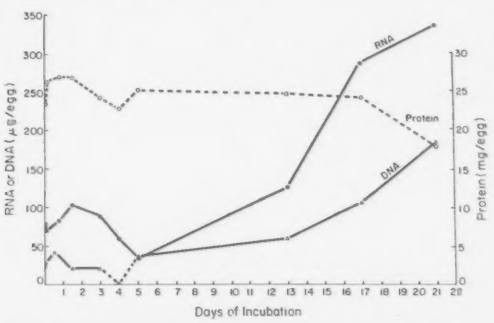

FIG. 1. DNA, RNA, and protein concentration per egg of rainbow trout (*Salmo gairdneri*) during different prehatching stages. DNA concentration on the 4th day of incubation was below the analytical sensitivity and the solid line of the curve between the 3rd and 5th days of incubation is replaced with a dotted line.

Protein concentration per ontogenetic unit during the prehatching stages of development was essentially constant, and no significant differences were evident among the means until just prior to hatching, when protein concentration declined significantly (Fig. 1). These prehatching protein concentration data agree with the results of Hayes (1930); however, he reported a rise in the total protein just before hatching.

Protein retention in the fry or larva was calculated by dividing the concentration of protein in either stage by the protein originally stored in the unfertilized egg (Table 2). Protein metabolized or lost by hatching or by postyolk absorption was estimated by subtracting the protein retained at these stages from that in the unfertilized oocyte. Of the protein present in the unfertilized egg, 19.4% was metabolized or lost at hatching, while 49.1% was lost by postyolk absorption. Of the protein remaining in the yolk at hatching, 50.0% had been metabolized 14 days

TABLE 3. Free amino acid (FAA) concentrations at different stages of development of rainbow trout (*S. gairdneri*). SE = pooled standard error of mean; $n = 3$. *OU* = ontogenetic unit, defined as one egg or the developmental stage originating from one egg.

FAA	Stage no. 1	2	3	7	8	9	11	12	13	±SE
	(*nmol/OU*)									
Lys	57	21	17	47	46	63	210	40	951	75
His	36	10	10	29	34	49	138	26	636	36
Arg	43	9	6	32	35	60	182	34	751	74
Asp	546			433	319	162	163	26	417	52
Thr	49	176	155	34	26	28	105	17	467	44
Ser	113			96	52	87	265	48	570	47
Glu	371	98	91	310	261	301	227	37	651	62
Pro	27	9	—	19	27	42	152	34	651	57
Gly	51	16	15	36	45	48	163	29	801	55
Ala	140	39	36	78	79	83	210	46	958	80
Cys	36	14	13	34	28	23	402	5	1082	59
Val	110	38	30	79	72	73	34	35	710	8
Met	23	10	8	13	8	11	70	3	400	30
Iso	72	29	28	57	54	53	155	24	551	48
Leu	83	42	38	57	60	62	241	37	1128	83
Tyr	21	8	9	18	29	44	126	21	461	24
Phe	28	15	14	24	47	82	186	29	623	44
Total (*μmol/OU*)	1.8	0.6	0.5	1.4	1.2	1.3	3.0	0.5	11.2	0.7

later when yolk absorption was complete. Of the protein in the unfertilized egg, approximately 20% was retained in the fry at hatching and approximately 50% was retained in the larva immediately after yolk absorption.

During ontogenesis, concentrations of free amino acid (FAA) were highest in the unfertilized egg, followed by a marked drop ½ h after fertilization (Table 3). This concentration increased to near initial values in the late blastula and was nearly doubled in the yolk plus fry at hatching. Separation of the fry from the yolk revealed that only 17% of FAA at this stage was present in the fry. When yolk absorption was complete (14 days later) the larva contained 22 times more FAA than the fry at hatching. In unfertilized oocytes the acidic amino acids (aspartic acid and glutamic acid) were the most abundant. Of the neutral amino acids, serine, alanine and valine were present in appreciable concentrations. The high level of FAA in the unfertilized oocyte indicates that an appreciable protein turnover is taking place. The acidic amino acids were the highest in the FAA pool throughout the prehatching stages. On hatching cystine was highest, while at postyolk absorption cystine and leucine were the highest. Although impossible to define precisely, these values presumably represent the net effect of fluctuations in protein anabolic and catabolic reactions.

Discussion

Changes in total DNA, RNA, protein, and free amino acids are a measure of the metabolic activity during ontogenesis. The sequence of DNA increase or decrease followed by a like increase or decrease in RNA is presumed to be evidence of the dependence of RNA synthesis on the amount of DNA template available in the egg. Both DNA and RNA net synthesis in the egg was clearly evident at the end of 1 day of incubation, but it is not clear why DNA concentration dropped so precipitously from this point to an undetectable level at 4 days (early blastula). It is probable that during this period of ontogenesis catabolism is so great that only a decline in net concentration is evident using this finite procedure. In any case, RNA net concentration followed the same pattern of decline. The decline of RNA concentration was also reported by Dontsova et al. (1970) for trout eggs, but neither decline was observed by Hagenmaier (1969) in *S. gairdneri* and *S. trutta*. This could have happened because certain early stages in both studies were not sampled. From the 3rd or 5th days of

incubation, which correspond to the early and late blastula stages, DNA and RNA, respectively, rose until hatching.

DNA and RNA values obtained in this study are much higher than those values previously reported for trout eggs when yolk-free blastoderm were assayed (Hagenmaier 1969; Dontsova et al. 1970). Removal of the yolk seems to contribute greatly to the markedly lower concentrations of DNA. Baltus et al. (1968) demonstrated that DNA was located in the yolk of amphibian (*Xenopus laevis*) eggs in highly polymerized molecules and was also capable of serving as a primer in RNA polymerase reduction. Another explanation for the previously reported low RNA and DNA values might be the phospholipid extraction step that is included in various methods such as the Schmidt-Thannhauser procedure (1945) for RNA and DNA isolation (Shibko et al. 1967). Dontsova et al. (1970) values were 3–4 times lower in magnitude than those given by Hagenmaier (1969). Therefore, it is suspected that some of the differences in findings are a reflection of differences in sample preparation and assay technique (Grant 1965; Williams 1965; Dontsova et al. 1970).

The sudden decline of total FAA immediately after fertilization was accompanied by a slight increase in total protein. This response indicates either that some protein synthesis took place or that the rate of protein degradation declined. The increase of FAA to approximately the original concentration of the unfertilized ovum coincided with the stage (blastula) at which RNA concentration was reported to have begun rising after a sudden decline (Dontsova et al. 1970). Therefore, higher rates of yolk protein breakdown may be required to activate protein biosynthesis, as was confirmed with birds by Klein et al. (1962). Accordingly, the rate of protein synthesis in rainbow trout eggs is similar to that in loach (*Misgurnus fossilis*) eggs, in which the rate of protein synthesis intensified at the blastula stage (Neyfakh and Abramova 1974). Fish embryos develop at the expense of yolk protein degradation and the liberation of amino acids (Monroy et al. 1961) as a function of specific proteases similar to those found in amphibian eggs (Deuchar 1958). The constancy of total protein concentration during the prehatching stages of development was probably due to the translocation of yolk protein to the embryo without significant quantitative losses, as was concluded by Williams (1967); the substantial drop at hatching is attributed to protein combustion (Smith 1957).

Data presented in this paper show that the rainbow trout unfertilized oocytes were equipped with a complete protein biosynthesis system supporting ontogenesis to, at least, the blastula stage.

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Influence of Lipid Pool Size on Bioaccumulation of the Insecticide Chlordane by Northern Redhorse Suckers (*Moxostoma macrolepidotum*)[1]

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ROBERTS, J. R., A. S. W. DEFRIETAS, AND M. A. J. GIDNEY. 1977. Influence of lipid pool size on bioaccumulation of the insecticide chlordane by northern redhorse suckers (*Moxostoma macrolepidotum*). J. Fish. Res. Board Can. 34: 89–97.

An effect of the lipid pool size on the accumulation and clearance of *cis*- and *trans*-chlordane by northern redhorse suckers (*Moxostoma macrolepidotum*) has been demonstrated. A technique was developed using ^{203}Hg-labeled methylmercury as a tracer, which permits the direct determination of the assimilation efficiency from food and the biological half-life of chlorinated hydrocarbons in terms of individual fish. Tissue retention of the insecticide chlordane in fish was directly proportional to the adiposity of the fish. For a fish containing 2% lipid, the half-life of the *cis*-isomer was 60 days and for the *trans*-isomer, 33 days. The net assimilation efficiency from food to fish tissue was also influenced by adiposity. These results on uptake and clearance, when used in a simple model for pollutant bioaccumulation, indicate that the steady state concentration of chlordane in fish will not exceed the concentration of chlordane in the diet unless the adiposity of the fish is high.

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Les auteurs démontrent un effet de la grandeur du pool lipidique sur l'accumulation et le coefficient d'épuration du chlordane des formes *cis* et *trans* chez des suceurs rouges (*Moxostoma macrolepidotum*). Ils ont développé une technique utilisant le mercure-méthyl marqué au ^{203}Hg comme traceur, qui permet la détermination directe de l'efficacité d'assimilation de la nourriture et la période biologique des hydrocarbures chlorés en terme de poissons individuels. La rétention par les tissus de l'insecticide chlordane dans le poisson est directement proportionnelle à l'adiposité du poisson. Chez un poisson contenant 2% de lipides, la période de l'isomère *cis* est de 60 jours, et celle de l'isomère *trans* de 33 jours. L'adiposité affecte également l'efficacité d'assimilation nette de nourriture dans les tissus du poisson. Ces données sur l'assimilation et le coefficient d'épuration, lorsque utilisées dans un modèle simple de bioaccumulation des polluants, indiquent que la concentration d'équilibre du chlordane chez les poissons ne dépassera pas la concentration du chlordane dans le régime à moins que l'adiposité du poisson ne soit élevée.

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IN addressing the problem of pesticide bioaccumulation in aquatic organisms, it can be assumed that food and water act as uptake vectors for pesticides found in fish. Branson et al. (1975) conclude that the tendency for an organic molecule to accumulate in aquatic organisms can be correlated with its lipophilicity. This is supported by evidence in earlier literature indicating that lipophilicity of a compound is an important factor influencing its transport across membranes and hence its bioassimilation (Wagner 1961; Schanker 1960). On this basis, lipophilic chemical pollutants including the active pesticidal components in commercial chlordane should be readily absorbed through gill and intestinal tissues with high assimilation efficiencies.

Lipophilic pesticides are associated with lipoidal tissues (Pocock and Vost 1974) and their metabolism and clearance should be directly related to the mobilization and turnover of tissue lipids (deFreitas and Norstrom 1974). The fractional turnover of the lipid pool should be related to lipid pool size and factors that control overall metabolic processes such as body size, temperature, and growth. These factors can be quite

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easily and reliably estimated even for fish in their natural environment. This is an extremely important consideration when modeling pollutant bioaccumulation since useful models should have general and easy applicability to real ecosystems and thus be able to use information derived from relatively unsophisticated, readily obtainable data.

The results presented in the present report form a quantitative definition of the bioaccumulation potential of *cis*- and *trans*-chlordane in terms of two variables: 1) its efficiency of uptake from food, and 2) its clearance from body tissues as functions of adiposity and body size. The methods used to study these variables involve the novel use of radiotracer techniques and represent a significant departure from previous methods. The development of the method and its advantages and limitations are described in the text. These results on chlordane have been used in conjunction with information published in the literature, recently reviewed by Norstrom et al. (1976), to develop a pesticide accumulation model designed to have general and easy applicability to fish.

Materials and Methods

Procedures for accurately estimating the assimilation efficiency of any compound from the gastrointestinal (G.I.) tract and its subsequent clearance from body tissues have generally involved the use of a large number of test animals. In one commonly used procedure, each test animal is given a single measured dose, administered directly into the stomach using a syringe or accurately calibrated perfusion pump. Groups of fish are then killed at various times after the dose and the body content of the compound in the tissues of individual fish is then estimated. The normal variations expected in assimilation efficiencies is greatly increased due to random regurgitation of the administered dose. The inability of this and other similar methods to yield consistently reliable values for the ingested dose is a serious disadvantage. What is needed is a simple procedure that permits accurate estimation of the amount of pollutant ingested by the fish and the quantity of pollutant initially sequestered in the tissues of each test animal, whether the animal was killed 1 day or 100 days after dosing. The procedure used in this study was developed to meet these demands.

In this procedure the administered dose must contain at least three functionally distinct components: a) carrier material (the regular diet or an individual dietary item like fat, protein, or carbohydrate); b) chemical of concern (either man-made or natural toxicant); c) indicator chemical (as tracer and quantitative reference for the chemical of concern). The indicator chemical should exhibit the following characteristics: chemical stability in the G.I. tract, biochemical stability in body tissues, and a high consistent assimilation efficiency. It should be eliminated more slowly than the chemical of concern. Assay methods for both chemicals should be reliable and simple and not interfere with each other. Besides these characteristics, an ideal indicator chemical should be readily available in a radionuclide-labeled form, preferably a gamma emitter with a long half-life relative to its biological half-life.

In the present study we have employed ^{203}Hg-labeled methylmercuric chloride as the indicator chemical. Work in this laboratory and elsewhere has established that methylmercury meets the requirements (Macleod and Pessah 1973; Giblin and Massaro 1973; deFreitas et al. 1975). The rationale for the method and how it works is explained below.

The fish are given the contaminated diet and after 3–5 h, a number are chosen at random and isolated in a separate tank. These fish will be referred to as Group I and the remaining fish as Group II. Group I is used to determine the assimilation efficiency of the indicator chemical and its clearance from body tissues. These values for the indicator chemical must be known in order to calculate the corresponding values for the chemical of concern on an individual fish basis. Values for these variables are based on the amount of ^{203}Hg remaining in the whole body of live fish.

The assimilation efficiency of the indicator chemical is based on the initial post-dose series of whole body measurements which define the fast-clearing portion of the ingested dose of mercury (Goldstein and Elwood 1971). The clearance time for the fast-clearing fraction coincides with the voidance time of the G.I. tract and represents a clearance of that portion of the ingested dose of mercury not absorbed by intestinal epithelium (deFreitas et al. 1975). The size of the slow-clearing compartment relative to the size of the ingested dose is therefore an effective measure of net assimilation efficiency, i.e. a measure of the fraction of the ingested dose sequestered in the tissues.

Clearance of the indicator chemical from body tissues of each fish in Group I, i.e. the slow compartment, is determined from the series of whole body measurements obtained during the post-dose period.

The method used to calculate the net assimilation efficiency of the chemical of concern is defined by the following equation.

$$A_{cc} = \frac{[\mu g_{cc}/(\text{counts/min})_{ic}]\text{in tissue} \times A_{ic}}{[\mu g_{cc}/(\text{counts/min})_{ic}]\text{in dose}} \quad (1)$$

where A_{cc} and A_{ic} are the net assimilation efficiency of the chemical of concern, *cc*, and the indicator chemical, *ic*, respectively. Values for the quantity of *ic* and *cc* are determined on individual tissue samples of a small subgroup of fish selected at random from Group II. The killing time for this subgroup is made to coincide with the voidance time of the ingested dose of contaminated food.

This approach to the problem of estimating the net assimilation efficiency from the G.I. tract represents a simplification of the two compartment model proposed by Goldstein and Elwood (1971). The competing processes of absorption and initial egestion of the ingested material are taken into account. Hence,

this measure of the assimilation efficiency is particularly useful in modeling the bioaccumulation process and estimating the steady state bioconcentration factor of chlordane and other persistent pollutants. If clearance of the chemical of concern from the slow compartment is relatively rapid, these values may be adjusted for any significant post-dose clearance.

In the present method clearance of the chemical of concern from individual fish is based on the difference between its content in tissues determined at death and the estimated quantity initially sequestered in the tissues. The amount of the chemical of concern and indicator chemical remaining in the tissues of each fish when killed is determined by direct analysis. The actual quantity of contaminated ration ingested, R, can be calculated from the following equation:

$$R = \frac{B_{ic}^{s} \times F}{C_{ic}^{R} \times A_{ic}} \quad (2)$$

where B_{ic}^{s} is the body content of the indicator chemical at the time of death, F is a correction factor for loss of the indicator due to clearance, C_{ic}^{R} is the concentration of the indicator chemical in the ration, and A_{ic} is the assimilation efficiency of the indicator chemical.

The amount of the chemical of concern ingested by each fish, D_{cc}, is:

$$D_{cc} = R \times C_{cc} \quad (3)$$

where C_{cc} is the concentration of the chemical of concern in the dose material. The amount of the chemical of concern initially sequestered in the fish tissues, B_{cc}^{i}, is estimated by multiplying the amount ingested, D_{cc}, by its assimilation efficiency, A_{cc}, i.e.

$$B_{cc}^{i} = D_{cc} \times A_{cc} \quad (4)$$

Clearance of the chemical of concern can now be analyzed in terms of the fraction of the initial quantity sequestered in the tissues and the fraction remaining in the tissues at death.

Chemicals

An experimental formulation (designated by Velsicol Chemical Company as HCS-3260) contained approximately a 3:1 mixture of the *cis*- and *trans*-isomers of chlordane (1,3,4,7,8,9,10,10-octachloro-*endo*-tricyclo[5.2.1.$0^{2,6}$]dec-8-ene) in >95% purity and was used without further purification. ^{203}Hg-labeled methylmercuric chloride (specific activity ≃ 1 mCi/mg Hg) was obtained from New England Nuclear. All solvents were pesticide grade and the ^{203}Hg-labeled methylmercury contained >2% of ^{203}Hg-labeled impurities as measured by the procedure of Westoo (1973). MS-222® (ethyl *m*-aminobenzoate methanesulfonate) was obtained from Sandoz (Canada) Ltd. and used as an anesthetic and killing agent.

Tissue Extraction

The fish were killed with an overdose of MS-222®, weighed and stored at −26°C prior to lipid extraction. The procedures outlined by deFreitas and Norstrom (1974) were used with only minor modifications for the preparation of the tissues for homogenation and subsequent total lipid extraction by the Bligh and Dyer (1959) procedure. The contents of the G.I. tract were isolated using a 1–2-ml hexane wash and then treated in a manner analogous to the other tissues. Samples of the homogenate (3–5 g) were used directly for ^{203}Hg analysis. *Cis*- and *trans*-chlordane were isolated from the lipid with a >95% recovery by column chromatography using the 5% deactivated Florisil column (PR grade, Floridin Corp.) described by deFreitas and Norstrom (1974). *Cis*- and *trans*-chlordane were eluted in the first 200 ml of *n*-hexane.

Chemical Analysis

Quantitative analysis for *cis*- and *trans*-chlordane was carried out on a Hewlett-Packard 5700 gas chromatograph equipped with a ^{63}Ni electron-capture detector and a 183 cm × 4 mm ID glass column packed with 1.5% SP 2250/1.95% SP 2401 on 80/100 mesh spelcon AW-DMCS support. The column was maintained at 200°C and the flow of carrier gas, nitrogen, was maintained at 60 cm^3/min.

Whole body clearance of mercury in live fish was measured by radiotracer techniques. Individual fish were held in a cylindrical plexiglass container filled with water. The cylinder was placed between two vertically opposing 5 cm diameter NaI crystals connected to an Ortec single channel analyzer (model #406A) and the usual array of equipment used in the detection and counting of gamma emissions. The cylinder was positioned in a defined location midway between the detector crystals to ensure a constant counting geometry. For a more detailed account of the precautions necessary in applying this technique, see Ruohtula and Miettinen (1975). The ^{203}Hg content of subsamples of tissue homogenate was determined using an automatic Nuclear Chicago counter, model No. 4233.

Protocols

Northern redhorse suckers (*Moxostoma macrolepidotum*) and white suckers (*Catostomus commersoni*) obtained from a local fish supplier were maintained in 100-ℓ fiberglass tanks equipped for continuous flow. The fish were grouped according to size and species. The density within individual tanks did not exceed 20 g/ℓ. At all times, the flow of dechlorinated water to each tank was maintained between 1 and 1.5 ℓ/min and the water temperature was maintained at 11°C ± 2. The tanks were drained and cleaned daily. All fish were acclimated for 3 wk to a maintenance diet of No. 2 Purina trout chow. Feeding regimes varied and are specified below.

A total of 64 northern redhorse suckers (*M. macrolepidotum*) weighing between 24 and 398 g (Table 1) were divided into groups according to size and acclimated to rations adjusted to the estimated metabolic requirements at maintenance of each group, using

TABLE 1. Chlordane (*cis*-, *trans*-, total) and ^{203}Hg in tissues of northern redhorse suckers (*Moxostoma macrolepidotum*) as a function of adiposity and postexposure time.[a,b]

Elapsed time (*days*)	Lipid content (%)	Fish size (*wet wt, g*)	Ratio of		
			Isomers	*cis*-chlordane (μg) to ^{203}Hg[d] (*counts/min*) × 10^{-4}	Total chlordane (μg) to ^{203}Hg[d] (*counts/min*) × 10^{-4}
0	5.2	50	0.27	3.47	4.40
	1.3	28	0.19	2.63	3.12
	0.9	47	0.23	2.96	3.64
	4.3	33	0.26	4.40	5.54
	3.4	40	0.27	2.93	3.72
	1.3	164	0.27	3.16	4.01
	0.9	218	0.26	2.36	2.97
	2.7	195	0.27	5.06	6.42
	0.3	61	0.27	2.49	3.16
	1.3	78	0.25	3.87	4.83
	0.3	61	0.23	2.36	2.90
	0.8	79	0.28	2.94	3.76
	3.3	81	0.29	4.02	5.18
5	1.5	30	0.19	2.98	3.54
	1.4	24	0.23	2.84	3.49
	1.8	31	0.23	4.55	5.59
	3.3	30	0.30	3.99	5.54
17	1.9	236	0.23	3.20	3.93
	1.6	74	0.20	2.15	2.58
	2.2	82	0.27	2.44	3.09
	1.6	36	0.18	2.68	3.16
	1.4	52	0.14	2.85	3.24
	1.5	27	0.19	2.15	2.55
	1.4	35	0.20	2.71	3.25
	1.2	42	0.20	1.72	2.06
	1.3	43	0.14	1.64	1.86
	1.7	30	0.20	3.27	3.92
37	2.7	104	0.14	3.78	4.30
	2.1	171	0.13	4.25	4.80
	2.1	83	0.22	3.63	4.42
	1.7	44	0.19	2.63	3.12
	2.2	57	0.22	3.90	4.75
	2.0	56	0.21	3.22	3.89
	2.4	69	0.22	3.73	4.55
	1.8	80	0.21	2.55	3.08
	1.0	64	0.16	1.31	1.51
	2.8	108	0.25	0.96	1.20
	2.9	44	0.10	2.23	2.45
	2.1	31	0.22	4.29	5.23
	3.3	59	0.21	4.23	5.11

estimates of Winberg (1956). Rations were established which provided a maintenance diet throughout the acclimation period and the post-dose period.

The fish were dosed on 5 consecutive days and during this period the fish were fed at the rate of 1.5% body wt/day. The ration contained 45 μg chlordane/g of dry feed (determined by GLC analysis), and 0.4 μg (0.4 μCi) ^{203}Hg-methylmercury/g of dry feed. The ratio of *trans*- to *cis*-isomer in the diet was 0.36. Eighteen fish were selected at random from the 64 dosed fish. The assimilation efficiency of methylmercury and its clearance from body tissues was estimated on this subgroup of 18 fish, on the basis of whole body ^{203}Hg levels measured in each fish on day −6, −2, 0, 9, 29, 50, and 55. Throughout this paper day 0 refers to the 1st day fish were killed for chlordane analysis, i.e. 11 days after the 5-day dosing regime was initiated and 6 days post-dose. All fish in this subgroup were killed on day 56 and their tissues analyzed for chlordane, ^{203}Hg, and lipid. The remaining fish from the original group of 64 dosed fish were killed on day 0, 5, 19, and 37 and their tissues were also analyzed for chlordane, ^{203}Hg, and lipid.

In a separate test to check the uptake of methyl-

TABLE 1. (*Concluded*)

Elapsed time (*days*)	Lipid content (%)	Fish size (*wet wt, g*)	Ratio of		
			Isomers	*cis*-chlordane (μg) to $^{203}Hg^{d}$ (*counts/min*) × 10^{-4}	Total chlordane (μg) to $^{203}Hg^{d}$ (*counts/min*) × 10^{-4}
56	1.2	398	0.07	1.08	1.15
	3.2	251	0.23	4.14	5.09
	1.3	236	0.08	1.06	1.15
	1.0	122	0.06	2.63	2.78
	1.5	101	0.08	2.16	2.33
	1.8	133	0.15	2.51	2.88
	1.9	24	0.16	2.05	2.39
	2.6	40	0.20	2.86	3.43
	0.9	24	0.09	0.82	0.89
	1.1	82	0.09	0.70	0.76
	2.2	38	0.15	2.53	2.90
	2.7	78	0.20	4.50	5.40
	1.6	79	0.09	1.92	2.09
	1.6	99	0.14	2.55	2.90
	1.1	58	0.13	2.90	3.27
	0.8	49	0.07	1.53	1.63
	0.9	30	0.09	0.65	0.71
Chlordane in dose	—	—	0.37	6.75	9.24
Tissue spiked homogenate	—	—	0.36	—	—
Chlordane standard	—	—	0.36	—	—

[a]Whole body wet weight basis.

[b]The weights of the fish in the group killed on day 56 were monitored throughout the experiment and generally did not vary by more than 6%.

[c]Elapsed time assigning day 0 as the 6th day after the dosing regime was completed.

[d]Assumes that no appreciable biological clearance occurred over the observation period, since clearance of ^{203}Hg > 5% was not observed. Measurements were corrected for physical decay in order for the values to be related to the amount ingested.

mercury from spiked rations, a group of five northern redhorse suckers (*M. macrolepidotum*) weighing between 16 and 278 g, were starved for 5 days and then allowed to feed freely for 3.5 h on a ration spiked with 100 μg/g of methylmercury. The excess feed was then removed and the tanks were cleaned. During the post-dose period the fish were fed ad libitum. Whole body ^{203}Hg levels of the individual live fish were determined immediately after the fish were removed from the contaminated feed and after 24, 144, and 216 h (Table 2).

The net assimilation efficiency of chlordane was also determined by gastric intubation of a chlordane-spiked corn oil solution in the absence of methylmercuric chloride. Four white suckers (*C. commersoni*) weighing from 171 to 223 g were starved for 1 day. A 0.125-g aliquot of corn oil solution containing 340 μg of chlordane was then introduced by intubation into the stomach of each fish using a Harvard perfusion pump. One hour after the dose was administered and each day thereafter the fish were fed ad libitum. The fish were killed 120 h post-dose for analysis of individual tissues.

Results and Discussion

ELIMINATION OF CHLORDANE

Persistence of the chlorinated hydrocarbon, chlordane, in tissues of the northern redhorse suckers (*M. macrolepidotum*) was directly related to the lipid content of the individual fish (Table 1). Field and laboratory studies had previously suggested that an adiposity factor could influence the accumulation of chlorinated hydrocarbons by fish (Harvey et al. 1971; Hamelink et al. 1971). However, previous laboratory studies on the dynamics of such compounds in aquatic organisms have generally used pooled samples and the reported data do not have sufficient resolution to elucidate any such trends. The relative quantity of the *trans*- in comparison with the *cis*-isomer generally decreased progressively as the fish were killed over the 56-day post-dose (Table 1). This shift tended to be more pronounced in the leaner fish. Linear regression analysis of these data

TABLE 2. Assimilation efficiency of methylmercury from the gastrointestinal tract of northern redhorse suckers.

Fish size (*wet wt, g*)	Ingested amount of ^{203}Hg (*counts/min*)[a]	Estimated amount of ^{203}Hg deposited in tissues (*counts/min*)[a]	Net assimilation coefficient
278	12,947	8,606	0.66
195	7,637	4,991	0.65
25	1,655	1,042	0.63
19	2,662	2,070	0.77
16	1,742	1,338	0.76

[a]Assumes that no appreciable clearance from the tissues occurred over the 216-h observation period, since clearance of ^{203}Hg > 5% was not observed in the fish monitored for 56 days. Measurements were corrected for physical decay in order for the values to be related to the amount ingested.

indicated that the decrease in the ratio of the two isomers correlates well, $r = 0.82$, assuming that the logarithm of the ratio is inversely proportional to the percent lipid of the individual fish, L (whole body wet weight basis), and is proportional to the elapsed time, t, in days, i.e.

$$\ln(\text{g, } \textit{trans}\text{-isomer/g, } \textit{cis}\text{-isomer}) = -1.39 - 0.019\, t\text{L}^{-1} \quad (5)$$

If the shift in isomer ratio is related to metabolic rate, the relationship could be formulated as follows:

$$\ln(\textit{trans}\text{-isomer}/\textit{cis}\text{-isomer}) = \text{z} - tw^{\alpha}\text{L}^{-1} \quad (6)$$

where z is a constant, w is the body weight in grams, and α is usually assigned a value of about −0.2 (Winberg 1956). Using our data with equation (6) and allowing the body weight exponent, α, to vary at random did not significantly improve the correlation. Given the value for α, the insensitivity of the clearance rate to changes in the body weight is not altogether unexpected. Going from a 50- to a 300-g fish would represent only a 30% decrease in clearance rate per gram of fish, and hence it would be difficult to demonstrate this relationship using our results from fish of relatively limited range in body weight.

In light of the number of studies on the metabalism of chlordane and the absence of any evidence that isomerization of the *trans*- to the *cis*-isomer occurs in living organisms, the shift in the ratio of the two chlordane isomers is best discussed in terms of the preferential metabolism and clearance of the *trans*-isomer from the tissues. Studies on the metabolism of chlordane are reviewed in NRCC (1974).

The chlordane to mercury ratios for the groups killed on days 37 and 56 (Table 1) show a similar pattern to the shift observed for the ratio of the isomers (Table 3). The half-life of *cis*-chlordane, based on these latter time points, was 30 days for a fish with a lipid content of 1%. If the data obtained on days 5 and 17 had been included in the regression analysis, the half-life would have been increased to about 38 days, but the strength of the correlation would have been significantly decreased. With lipophilic compounds like chlordane this is probably not due to a bimodal clearance phenomenon, but rather to an inherent problem in linear regression analysis of this type of data where a large portion of the time points are obtained during a period where clearance is too small to be detected above the normal scatter of the data.

Treatment of the clearance of the two isomers in terms of two independent first-order processes indicates that the following relation should obtain:

$$\ln(\text{isomer ratio}) = \text{z} - (k_{trans} - k_{cis})t \quad (7)$$

where z is a constant and k_{trans} and k_{cis} refer, respectively, to the clearance coefficients of the *trans*- and *cis*-isomers for a l-g fish with a 1% lipid pool. Accordingly, the difference between the clearance coefficients of the *trans*- and *cis*-isomers is estimated to be 0.019 d^{-1}L using equation (5) where d is time in days. The clearance coefficient of the *trans*-isomer, k_{trans}, is 0.042 d^{-1}L and of the *cis*-isomer, k_{cis}, is 0.023 d^{-1}L. The clearance of the *trans*-isomer is thus about 1.8 times faster than that of the *cis*-isomer.

Studies with birds and rats indicate that whole body clearance of polychlorinated biphenyls and DDT are directly proportional to the fractional turnover of lipid (deFreitas and Norstrom 1974; Pocock and Vost 1974). There is evidence in the literature (deFreitas and Norstrom 1974; Findlay and deFreitas 1971) that adiposity of the test organisms should be expressed in terms of total neutral lipid rather than total lipid since phospho-

TABLE 3. Regression analysis: the relation between adiposity and clearance of chlordane in northern redhorse suckers.

Relation	y intercept $\hat{\alpha}$	slope $\hat{\beta}$	r
ln (*trans*-:*cis*-chlordane)[a] vs. the ratio of the elapsed time to the % lipid, tL^{-1}	-1.39 ± 0.06	-0.0192 ± 0.002	-0.82
ln (*cis*-chlordane:^{203}Hg $\times$ 10^4)[b] vs. the ratio of the elapsed time to the % lipid, tL^{-1}	1.57 ± 0.18	-0.023 ± 0.005	-0.66
ln (total chlordane:^{203}Hg $\times$ 10^4)[b] vs. the ratio of the elapsed time to the % lipid, tL^{-1}	1.68 ± 0.18	-0.025 ± 0.004	-0.64

[a]Based on data presented in Table 1.
[b]Based on data collected on days 37 and 56, Table 1.

TABLE 4. Chlordane residues in white suckers (*Catostomus commersoni*) given a single introgastric dose[a] of chlordane.[b]

	Fish size (*wet wt, g*)			
	223	177	191	171
Lipid content				
wet wt % whole body	3.8	8.1	2.5	3.4
Lateral muscle				
g chlordane $\times$ 10^7	250	382	205	573
μg/g tissue	0.23	0.45	0.37	0.57
% total isolated	25	30	23	40
Remaining tissues				
g chlordane $\times$ 10^7	727	867	647	757
μg/g tissue	0.67	1.04	0.93	0.90
% total isolated	73	67	73	53
Liver				
g chlordane $\times$ 10^7	13	28	29	84
μg/g tissue	0.32	0.54	0.81	2.05
% total isolated	1.3	2.1	3.3	5.3
Gut contents				
g chlordane $\times$ 10^7	8	35	43	21
Total chlordane burden[c]				
g chlordane $\times$ 10^7	990	1277	881	1415
Fraction of ingested dose in tissues	0.40	0.51	0.35	0.57

[a]The size of the ingested dose was 2500 $\times$ 10^{-7} g *cis*-chlordane/fish.
[b]Calculated in terms of total *cis*-chlordane. The diet contained <150 μg/kg of chlordane. The *trans*-:*cis*-isomer ratio remained constant in all tissues at 0.28 $\pm$ 0.03. All fish were killed 120 h post-dose.
[c]Total of all tissues not including gut contents.

lipids do not appear to form part of the lipid pool involved in pesticide storage and release. These observations lend support to suggestions made in recent years (Hamelink et al. 1971, Harvey et al. 1971) that residue patterns of chlorinated hydrocarbons such as PCB's and DDT in aquatic fauna may be as much a function of the variation in the lipid content of the individual fish as of their specific food-web patterns.

ASSIMILATION FROM THE G. I. TRACT AND TISSUE DISTRIBUTION

When chlordane in corn oil solution (as carrier) was administered by gastric intubation, up to 57% of the dose was accounted for by the residues in body tissues (Table 4). Essentially similar values for net assimilation efficiency (Table 5) were obtained using the new method and 0.7 as the net assimilation coefficient for methylmercury (Table 2). Within 5 days post-dose, $<5\%$ of the dose administered by gastric intubation remained in the gut; 30–60% was absorbed into the tissues. At this time, the lateral muscle fillets accounted for 23–40% of the total body burden and the liver 5% or less. The ratio of the *trans*- to the *cis*-isomer in the homogenates of liver, lateral muscle, and the remaining tissues did

TABLE 5. Fraction of *cis*-chlordane absorbed from the diet[a] by northern redhorse suckers.

Tissue lipid (% whole body wet wt)	Net assimilation coefficient[b]
0.3	0.26
0.3	0.24
0.8	0.30
0.9	0.30
0.9	0.24
1.3	0.40
1.3	0.27
1.3	0.33
2.7	0.52
3.3	0.42
3.4	0.30
4.3	0.45
5.2	0.36

[a]From the fish sacrificed on day 0 (Table 1).
[b]Calculated as described in experimental section.

not vary as a function of tissue type. The isomer ratio decreased 20–30% in all the tissues of the initial groups killed 6 days post-dose when compared with the ratio present in the feed.

While these estimates of net assimilation indicate that chlordane is readily absorbed from the G.I. tract, they are low compared with the net assimilation coefficients determined for other chlorinated hydrocarbons. For example, assimilation coefficients for the uptake of dietary DDT, DDE, and PCB's by perch calculated from data reported by Sodergren (1973) yield values all > 0.7. Preliminary work in this laboratory showed that $> 80\%$ of an ingested dose of DDT was assimilated into the tissues of brown bullheads (*Ictalurus nebulosus*). However, the experimentally determined net assimilation coefficients reported in this study may reflect not only the extent to which a chemical is absorbed from the G.I. tract, but any losses that may occur prior to analysis. The low values for assimilation of chlordane ranging from 0.2 to 0.6 and the fact that these values appear related to the lipid pool size strongly suggest that an appreciable portion of the absorbed chemical may have been rapidly eliminated after its initial appearance in the circulatory system. The apparent influence of adiposity on the net assimilation of chlordane is consistent with the observation that the rate of clearance of either isomer of chlordane is inversely proportional to lipid pool size. These two factors could explain why fatter fish appear to retain a greater portion of the absorbed fraction of an ingested dose.

BIOCONCENTRATION FACTORS

The uptake of a pollutant, P, by fish may be described by the following equation:

$$\frac{d[P_{tissue}]}{dt} = \frac{A[P_{medium}]V}{w} \quad (8)$$

where A refers to the net assimilation coefficient for the uptake of the pollutant from either the water, A_{water}, or the diet, A_{feed}, V refers to either the daily ingestion rate, V_{feed} (grams per day) or daily respiration volume, V_{water} (grams per day). $[P_{medium}]$ and $[P_{tissue}]$ refer to the concentration of the pollutant in the exposure medium, water or feed, and the concentration of the pollutant in the tissues of the fish, respectively. A_{water} refers to the fraction of the pollutant in the water absorbed across the gills and generally believed to be > 0.75 for hydrocarbons like chlordane (Norstrom et al. 1975).

Assuming a single-compartment model and that first-order kinetics obtain, the clearance of a pollutant from the tissues can be described by the equation:

$$\frac{d[P_{tissue}]}{dt} = -kw^{\alpha}L^{-1}[P_{tissue}] \quad (9)$$

When the range in weight is small, the body weight term associated with the clearance function may be omitted. Such is the case in the present experiment. Combining equations (8) and (9), the overall accumulation of the *cis*-isomer of chlordane can be described by:

$$[P_{tissue}] = [P_{medium}]\frac{AVL}{kw} \times [1 - e^{(-k_{cis}\ tL^{-1})}] \quad (10)$$

where the rate constant, k, has units of d^{-1} and the concentration in the medium is constant.

The steady state concentration factor, CF_{ss}, i.e. when an equilibrium is established between the medium and the tissue, is given by the equation:

$$CF_{ss} = AVL/kw \quad (11)$$

Implicit in this derivation is the assumption that the fish are kept on maintenance diets and that the pollutant level in the exposure medium remains constant. In situations where the body size of the test organism changes, a growth dilution factor needs to be considered, since growth would tend to decrease the observed concentration factor. In the case of a 100-g fish (assuming $L = 1\%$) the predicted steady state concentration factor associated with the dietary intake of the *cis*-isomer of chlordane is approximately 0.1. At 12°C and assuming routine metabolism, V_{feed} is estimated to be 0.79 g/day (wet wt). A_{feed} for *cis*-chlordane is estimated from this study to be 0.4. As noted, this may be a conservative estimate. The comparable steady state concentration

factor associated with the water vector could be around 5.5×10^3. A_{water} is assumed to equal 0.75 and the daily respiration volume, V_w, at 12°C is estimated from Norstrom et al. (1975) to be 1.7×10^4 g water/day. These estimates suggest that, like other persistent chlorinated hydrocarbons, *cis*- and *trans*-chlordane could accumulate in the tissues of fish that inhabit contaminated ecosystems. It is important to note that food chain magnification by leaner fish is not indicated. Tissue levels of chlordane cannot significantly exceed those in the food unless the water vector is dominant.

Development of adequate approaches on which to estimate the bioaccumulation potential of lipophilic compounds should be predicated upon the consideration of at least the following physiological restraints: 1) adiposity, 2) weight of organism, 3) growth of organism, and 4) metabolic rate. It follows that the quantification of these factors should be included in any standardized testing protocols for the determination of steady state concentration factors. Possibly the simplest approach to developing data that are useful indicators of the bioaccumulation potential of a compound is to document the growth rate, lipid content, and the weight of the fish.

Acknowledgments

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Bicinctus-Group of the Genus *Cricotopus* Van der Wulp (Diptera: Chironomidae) in the Nearctic with a Description of a New Species

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OLIVER, D. R. 1977. *Bicinctus*-group of the genus *Cricotopus* Van der Wulp (Diptera: Chironomidae) in the Nearctic with a description of a new species. J. Fish. Res. Board Can. 34: 98–104.

Three species, *Cricotopus bicinctus* (Meigen), *C. vierriensis* Goetghebuer, and *C. mackenziensis* n.sp., in the *bicinctus*-group of the genus *Cricotopus* Van der Wulp are now known to occur in the Nearctic. The new species is described from the Fort Simpson area, Northwest Territories, and compared with a closely related species, *Cricotopus* (*s.s.*) *bicinctus*. Most of the differences between the two species are related to the smaller size of *C. mackenziensis*.

OLIVER, D. R. 1976. *Bicinctus*-group of the genus *Cricotopus* Van der Wulp (Diptera: Chironomidae) in the Nearctic with a description of a new species. J. Fish. Res. Board Can. 34: 98–104.

Trois espèces, *Cricotopus bicinctus* (Meigen), *C. vierriensis* Goetghebuer, et *C. mackenziensis* n.sp., faisant partie du groupe *bicinctus* du genre *Cricotopus* Van der Wulp sont présentement connues de la région néo-arctique. L'auteur décrit la nouvelle espèce des environs de Fort Simpson, Territoires du Nord-Ouest, et la compare avec une espèce étroitement apparentée, *Cricotopus* (*s.s.*) *bicinctus*. La plupart des différences entre les deux espèces sont liées à la taille plus petite de *C. mackenziensis*.

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HIRVENOJA (1973) included three species, *Cricotopus bicinctus* (Meigen), *C. pallidipes* Edwards, and *C. vierriensis* Goetghebuer, in the *bicinctus*-group of *Cricotopus* (*Cricotopus*) Van der Wulp. Of these species, only *C. bicinctus* had been previously reported from the Nearctic (Sublette and Sublette 1965; Hirvenoja 1973). In this paper new distributional records for *C. bicinctus* are given and the first occurrence of *C. vierriensis* in the Nearctic is reported.

During a study of the response of *C. bicinctus* to exposure to crude oil in the Fort Simpson area, Northwest Territories, (Rosenberg et al. 1977a), a fourth, previously undescribed species in the *bicinctus*-group was discovered. The adults, pupa and 4th instar larva of this taxon, *Cricotopus* (*s.s.*) *mackenziensis* n.sp., are described and compared with those of a sibling species, *C. bicinctus*. Data on the ecology of *C. mackenziensis* n.sp. and of *C. bicinctus* living in small rivers in the Fort Simpson are presented in Rosenberg et al. (1977b).

Measurements and ratios follow Schlee (1966) and Sæther (1969) except the maximum length of each leg segment was measured instead of length of the median axis. The wing length was measured as the distance from the arculus to the wing tip. The measurements and counts are presented as ranges followed by a mean ± 1 SD and the number of specimens in parentheses.

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Bicinctus Group

Diagnosis — Hirvenoja (1973) should be consulted for a more complete description and keys.

Male — Tergites I and IV light colored (abdomen of *C. pallidipes* unicolorous); fore tibia with light ring. Chaetotaxy of abdominal tergites reduced; on tergites III and IV median and lateral rows of setae uni- to biserial, posterior end of lateral rows extend medially but not joining medial row. Inner lobe of gonocoxite simple, more or less arched posteriorly.

Female — Color and abdominal chaetotaxy as in male. Spermatheca small (< 80 μm long), spherical; spermathecal ducts straight or twisted (in *C. pallidipes*).

Pupa — Abdominal segments II and III with distinct pedes spurii B; median and posterior spine

patterns on tergites III–VI separate (see Hirvenoja 1973, fig. 142_{13} and 144_{12}); posterior of 4 lateral setae on segment VIII smaller than other 3 setae. Three setae on each anal lobe of equal thickness.

Larva — Median tooth of hypostomium 2½–3 times as broad as one 1st lateral tooth. Inner margin of mandible with small spines, or smooth. Setae tufts on abdominal segments small, < ½ length of one segment.

Remarks — Three species, *Cricotopus bicinctus*, *C. pallidipes* and *C. vierriensis* were assigned by Hirvenoja (1973) to the *bicinctus* group of *Cricotopus*. To these a fourth species, *C. mackenziensis* n.sp., is added. The new species plus *C. bicinctus* and *C. vierriensis* form a well defined group, but *C. pallidipes* differs in several characteristics. The inclusion of *C. pallidipes* raises problems in defining the morphological characteristics of the group, e.g. the abdomen is unicolorous unlike the other three species. The species is based on only a few adult specimens; the immature stages are unknown. Hirvenoja (1973) states that the systematic position of *C. pallidipes* is uncertain and it will probably remain uncertain until the immature stages are discovered. However, *C. mackenziensis* n.sp. and *C. pallidipes* share one characteristic which tends to support Hirvenoja's placement of *C. pallidipes*. The dorsal edge ("crista dorsalis" of Hirvenoja 1973) of the gonostylus of the male hypopygium is angular in both species, whereas it is relatively straight in the other two species. In his key to the groups of the subgenus *Cricotopus*, Hirvenoja (1973) notes that if the abdomen has light markings then the "crista dorsalis" is absent. The abdomen of *C. mackenziensis* n.sp. is banded. Therefore, in the coloration of the abdomen and in the structure of the gonostylus *C. mackenziensis* n.sp. combines the character state of *C. bicinctus* and *C. vierriensis* on the one hand with the character state of *C. pallidipes* on the other.

Cricotopus (*s.s.*) *mackenziensis* n.sp.

Cricotopus (*s.s.*) *mackenziensis* is smaller in size but otherwise similar to *C. bicinctus*. *Cricotopus bicinctus* has been described in detail by Hirvenoja (1973, fig. 142_{1-13} and 143_{1-12}) therefore, only the differences between the two species are presented in the description below. The measurements and counts of some of the characters of both species are compared in Tables 1 and 2.

Male — Smaller than *C. bicinctus*; ranges and means of most characters presented in Table 1 lower than those of *C. bicinctus*, with little or no overlap in A.R., length of 4th and 5th palp segments, wing length, and length of most leg segments. Head — pair of small, cylindrical frontal tubercles (Fig. 3) on vertex near midline of head. Hypopygium — dorsal edge of gonostylus ("crista dorsalis" of Hirvenoja) angular; relatively straight in *C. bicinctus* (compare Fig. 1 and 2).

Female — Smaller than *C. bicinctus*; overlap in measurements and counts between species about the same as in males, except wing length shorter, 1.18–1.32 mm, 1.24 ± 0.04(14). Head — frontal tubercles more conical than in male and located midway between dorsal margin of eyes and midline of head. Legs — 1st tarsal segment of mid- and hindlegs with 7–10, 8.33 ± 1.03(6) and 5–9, 7.14 ± 1.57(7) curved sensory setae ("Sz" of Hirvenoja), respectively.

Pupa — Smaller than *C. bicinctus*. Cephalothorax — frontal, antepronotal, humeral and dorsocentral setae robust and black; mounted on low tubercles; posterior pair of dorsocentral setae closer to anterior pair than in *C. bicinctus* (compare Fig. 4 and 5); dc_3 situated distinctly anterior to dc_4. Prothoracic horn short (91–117 mm, 104.98 ± 7.50(19)), tip blunt, rarely pointed and without apical spines. Spines on dorsum of thorax coarse (Fig. 4). Abdomen — spine pattern on tergites II–VI same as in *C. bicinctus* but individual spines coarser.

Larva (*4th instar*) — Smaller; head capsule narrower than in *C. bicinctus* (Table 2). Head — antenna length and A.R. lower but with considerable overlap between the two species (Table 2). Hypostomium narrower with some overlap; median tooth about same width; ratio of hypostomium width to median tooth width lower but some overlap occurs (Table 2); color lighter than that of *C. bicinctus* and contrast between central area (median, 1st and 2nd lateral teeth) and lateral areas greater (compare Fig. 6 and 7). Mandible shorter (Table 2); outer margin smooth, without crenulations.

Data documenting differences between the 2nd and 3rd instars of the two species is presented in Rosenberg et al. (1977b).

Types — *Holotype* — Male, Harris River (61°52′N, 121°18′W), District of Mackenzie, Northwest Territories, 26.VII.1972 (FWI Pipeline Project); type No. 14,015 in the Canadian National Collection, Ottawa.

Paratypes — 31 males (1 with associated pupal and larval exuvia), 17 females, 30 pupae (14 with associated larval exuvia), and 19 larvae, with data given below: paratypes deposited in Cana-

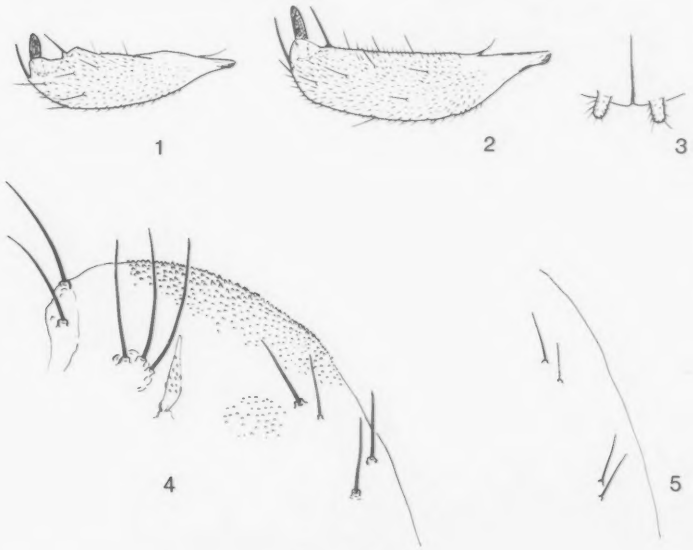
TABLE 1. Comparison of some characters of the males of *Cricotopus* (*s.s.*) *mackenziensis* n.sp. and *C.* (*s.s.*) *bicinctus* (Meigen).

	mackenziensis		*bicinctus* (from Hirvenoja 1973)	
Antenna				
A.R.	1.05–1.37;	1.16± 0.07 (16)	1.43–1.75;	1.57
Head				
Vertical setae	1–2;	1.36± 0.50 (19)	0–3;	1.4
Postorbital setae	1–4;	3.16± 0.90 (19)	3–7;	4.3
Clypeal setae	9–13;	10.26± 1.37 (19)	7–20;	13.6
Palp segments (μm)				
2	28–39;	33± 2.90 (18)	40–60;	50
3	52–75;	70± 5.80 (18)	70–95;	90
4	59–96;	78± 8.50 (17)	95–120;	110
5	96–150;	120±16.07 (15)	150–190;	170
Thorax				
Antepronotal setae	2–5;	3.25± 0.93 (16)	3–8;	4.4
Acrostichal setae	8–13;	10.67± 1.18 (15)	13–26;	16.7
Dorsocentral setae	10–17;	13.18± 1.74 (17)	10–20;	13.9
Prealar setae	2–4;	3.0 ± 0.36 (16)	2–7;	3.5
Supra-alar setae	0–1;	0.36± 0.50 (14)	1–2;	1.1
Scutellar setae	5–10;	7.13± 1.60 (15)	6–12;	9.1
Wing				
Length (mm)	1.07–1.6;	1.31± 0.11 (18)	1.6–2.5;	2.0
V.R.	1.10–1.16;	1.13± 0.01 (19)	1.06–1.16;	1.12
R-setae	0–1;	0.10± 0.32 (19)	1.4;	1.9
R_1-setae	0;	0± 0 (19)	0	
R_{4+5}-setae	0;	0± 0 (19)	0	
Squamal setae	4–9;	5.28± 1.18 (19)	7–16;	10.7
Legs				
L.R.-Foreleg	0.53–0.62;	0.56± 0.02 (16)	0.55–0.66;	0.60
Midleg	0.42–0.49;	0.45± 0.02 (17)	0.45–0.50;	0.47
Hindleg	0.51–0.58;	0.54± 0.02 (16)	0.51–0.58;	0.54
Segment length (μm)				
Foreleg-F	450–550;	487±25.92 (17)	550–840;	710
T	550–690;	605±34.66 (17)	670–1080;	880
Ta_1	300–430;	335±28.75 (16)	400–640;	530
Ta_2	190–270;	217±18.15 (16)	240–400;	320
Ta_3	130–190;	149±13.89 (16)	170–290;	230
Ta_4	90–140;	106±10.88 (16)	130–200;	170
Ta_5	70–90;	73± 6.02 (16)	90–140;	110
Midleg-F	410–590;	488±36.76 (14)	570–870;	740
T	460–590;	507±30.57 (17)	590–890;	770
Ta_1	200–290;	230±21.50 (17)	280–450;	370
Ta_2	120–160;	131±10.29 (17)	170–250;	220
Ta_3	80–110;	94± 7.02 (17)	120–190;	160
Ta_4	50–80;	56± 7.86 (17)	80–120;	100
Ta_5	50–70;	57± 5.88 (17)	70–120;	100
Hindleg-F	430–540;	472±27.57 (15)	560–890;	720
T	500–670;	562±42.50 (16)	670–1070;	870
Ta_1	260–370;	300±25.82 (16)	370–550;	470
Ta_2	140–200;	162±13.42 (16)	200–330;	260
Ta_3	110–160;	131±12.36 (16)	170–270;	210
Ta_4	60–90;	66± 8.14 (16)	90–160;	120
Ta_5	50–80;	61± 5.74 (16)	80–140;	110
"SZ" Mid	0–1;	0.14± 0.37 (7)	0	
Hind	2–10;	4.62± 2.56 (8)	8–10	

TABLE 2. Comparison of some characters of the 4th instar larva of *C. (s.s.) mackenziensis* n.sp. and *C. (s.s.) bicinctus* (Meigen).

	mackenziensis		*bicinctus*	
Head width (μm)[a]	205–263;	240±11 (438)	268–374;	297±17 (280)
Antenna				
Length (μm)	79–106;	86±6.78 (18)	78–110;	98±8.27 (15)
A.R.	1.50–1.87;	1.68±0.11 (18)	1.63–2.19;	1.86±0.17 (15)
Hypostomium				
Basal width (μm)	88–119;	106±6.77 (22)	114–143;	129±6.99 (19)
Median tooth width (μm)	23–28;	26±1.59 (22)	20–31;	27±2.55 (18)
Hypostomium width: median tooth width	3.50–4.78;	4.16±0.35 (22)	4.00–6.12;	4.78±0.55 (18)
Mandible				
Length (μm)	117–135;	127±4.39 (22)	143–163;	155±5.88 (17)

[a]Head width measurements from D. Rosenberg (personal communication).

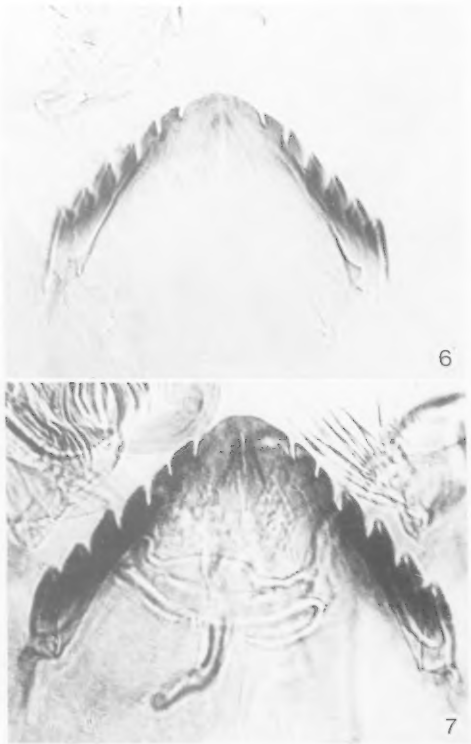


FIGURES 1–5

FIG. 1. *Cricotopus (s.s.) mackenziensis* n.sp., male, gonostylus; FIG. 2. *C. (s.s.) bicinctus* (Meigen), male, gonostylus (same scale as Fig. 1.); FIG. 3. *C. (s.s.) mackenziensis* n.sp., frontal tubercles; FIG. 4. *C. (s.s.) mackenziensis* n.sp., pupa, lateral view of cephalothorax; FIG. 5. *C. (s.s.) bicinctus* (Meigen), pupa, dorsocentral setae (same scale as Fig. 4).

dian National Collection, Ottawa; Freshwater Institute Collection, Winnipeg; J. E. Sublette Collection, Portales, N.M.; the United States National Museum of Natural History, Washington, D.C.; and Academy of Natural Sciences, Philadelphia.

NORTHWEST TERRITORIES, DISTRICT OF MACKENZIE: 1 lar., Harris River, 16.VIII.1971 (FWI Pipeline Project); 2♂♂, 2♀♀, Harris River, 18.VIII,1972 (A. P. Wiens); 1♂, Jean Marie Creek, 19.VI.1972 (FWI Pipeline Project); 2♂♂, Jean Marie Creek, 27.VI.1972 (FWI Pipeline Project); 2♂♂, Jean Marie Creek, 14.VIII.1972 (FWI Pipeline Project); 1♂, Jean Marie Creek, 29.V.1973 (D. R. Oliver); 2 lar., Martin River, 20.VII.1972 (A. P. Wiens); 2 lar., Martin River, 20.VII.1972 (FWI Pipeline Project); 1 lar., Martin River, 8.VIII.1972 (FWI Pipeline Project);



FIGURES 6–7

FIG. 6. *C. (s.s.) mackenziensis* n.sp., larva, hypostomium; FIG. 7. *C. (s.s.) bicinctus* (Meigen), larva, hypostomium (same scale as Fig. 6).

2♂♂, Martin River, 15.VIII.1972 (A. P. Wiens); 5 lar., Martin River, 18.VIII.1972 (FWI Pipeline Project); 1♂, 2♀♀, Martin River, 23.VIII.1972 (A. P. Wiens); 1♂ (pup. and lar. ex.), Martin River, 4.VI.1973 (G. Collins); 1♂, Trail River, 23.VI.1972 (A. P. Wiens); 1♂, 3♀♀, Trail River, 28.VI.1972 (A. P. Wiens); 16♂♂, 5♀♀, Trail River, 21.VII.1972 (A. P. Wiens); 1 pup. (lar. ex.), 1 pup., 1 lar., Trail River, 11.VIII.1972 (FWI Pipeline Project); 4♀♀, Trail River, 24.VIII.1972 (A. P. Wiens); 1♀, Trail River, 28.VIII.1972 (A. P. Wiens); 1♂, 1 lar., Trail River, 7.IX.1972 (A. P. Wiens); 6 pup. (1 with lar. ex.), Trail River, 7.IX.1972 (FWI Pipeline Project); 21 pup. (12 with lar. ex.), 6 lar., Trail River, 14.VI.1973 (FWI Pipeline Project); 1 pup. (with lar. ex.) Trail River, 9.VIII.1973 (FWI Pipeline Project).

Remarks The shorter wing length (Table 1) will separate most male specimens, and all females, of *C. mackenziensis* from those of *C. bicinctus*. Difficulty is encountered only in separating the larger male specimens of *C. mackenziensis* from the smaller male specimens of *C. bicinctus*. However, all males of *C. mackenziensis* may be distinguished by the presence of frontal tubercles and by having the gonostylus with an angular dorsal margin. In addition to a shorter wing length and presence of frontal tubercles the female of *C. mackenziensis* has a smaller number of curved sensory setae on the first tarsal segment of the mid and hind legs than has the female of *C. bicinctus*. The pupae and larvae of the two species may be distinguished with relative ease. The presence of robust black setae on the cephalothorax of the pupae of *C. mackenziensis* is distinctive. The larvae of *C. mackenziensis* have a narrower head capsule and shorter mandible than that of *C. bicinctus* (Table 2). Also the contrast in color between the central and lateral areas of the hypostomium is stronger in *C. mackenziensis* then in *C. bicinctus* (Fig. 6 and 7).

FIG. 8. Distribution of *C. (s.s.) bicinctus* (Meigen); (●) specimens examined, (▲) literature records.

Cricotopus (*s.s.*) *bicinctus* (Meigen)

Chironomus bicinctus Meigen 1818: 41
Cricotopus (*Cricotopus*) *bicinctus*: Hirvenoja 1973: 235

All the specimens listed below under "Specimens Examined" agree closely with Hirvenoja's (1973) description of *Cricotopus* (*s.s.*) *bicinctus*. The only observed difference is that two adult males have an A.R. lower than 1.43 (minimum 1.31). Differences between this species and *C.*

mackenziensis have been discussed under the latter.

Distribution — *Cricotopus* (*s.s.*) *bicinctus* is widespread in Europe (Hirvenoja 1973) and in North America (Fig. 8). It has also been recorded from Afghanistan (Hirvenoja 1973). In addition to the localities shown in Fig. 8, Beck and Beck (1959) have recorded this species from Florida, but no precise localities were given. Sublette and Sublette (1965) also record it from Michigan.

Specimens examined — Canada: YUKON TERRITORY: 3 lar., Caribou Bar Creek, 15.VIII.1971, 19.VIII.1971, 14.IX.1971 (FWI Pipeline Project). DISTRICT OF MACKENZIE, NORTHWEST TERRITORIES: 4♂♂, 1♀, 2 pup., Harris River, 18.VIII. 1972 (A. P. Wiens), 26.VII.1972, 15.VIII.1974, 22.VIII.1974 (FWI Pipeline Project); 1♂, 5 lar., Martin River, 18.IX.1971 (A. P. Wiens and C. B. Crocker), 20.VI.1972, 18.VIII.1972, and 23.VIII.1972 (FWI Pipeline Project); 2♂♂ (1 reared),[1] Rabbitskin River, 6.VI.1973 (D. R. Oliver), 11.VI.1973 (FWI Pipeline Project); 1♂, 1♀ (reared), 8 lar., Trail River, 6.VIII.1971, 9.VI–7.IX.1972, 7.IX.1972, 14.IX.1972 (A. P. Wiens), 7.IX.1972, 14.VI.1973 (FWI Pipeline Project). DISTRICT OF KEEWATIN, NORTHWEST TERRITORIES: 1♂ Baker Lake, 8.VIII.1949. ALBERTA: 3♂♂, Bigoray River, 21.VI.1973, 25.VIII. 1973 (H. Boerger); 1♂, Scandia, 9.VII.1956 (O. Peck). SASKATCHEWAN: 1♂, Maple Creek, 28.V. 1955 (J. R. Vockeroth). MANITOBA: 1♂, 2½ mi off Horse Island, Lake Winnipeg, 28.VII.1969 (S. S. Chang; FWI); 3♂♂ (reared), Calder's Dock, Lake Winnipeg, 11–17.VI.1971, 11–19.VI. 1971 (E. Johnson and M. P. McLean; FWI); 17–19.VI.1971 (M. P. McLean and M. Roberts; FWI); 1♀ (reared), Washaw Bay, Lake Winnipeg, 5–10.VIII.1971 (N. Hooper and E. Johnson; FWI). ONTARIO: 1♂ (reared), Brown's Bay, St. Lawrence River, 25.VI.1966 (J. R. Vockeroth); 1 pup. (lar. ex.), 1 lar., Butler Creek, Kawartha Lakes area, 2.VI.1970, 8.VI.1970 (D. L. Galloway); 1♂, Grenadier Island, St. Lawrence Islands National Park, 22.V.1975 (M. E. Roussel); 2♂♂, Jock River, 9.VI.1975 (D. R. Oliver); 1♂, Ottawa, 21.X.1971 (J. A. Downes); 1♂, (pup. ex.),[1] 1 pup. (lar. ex.), Ottawa River near Ottawa, 9.VII.1973 (M. Dickman). NEW BRUNSWICK: 2♂♂, Hayden Brook, 19.VII.1972 (Nashwaak Experimental Watershed); 1♂, N.W. Miramichi River, 22.VII.1962 (J. Marshall). NEWFOUNDLAND: 1♂, Astray Lake, 22.VIII.1957 (D. R. Oliver); 2 pup., 3 lar., Baie Vert River, 22.VI. 1971, 23.VI.1971 (Frost); 1 lar., Dillon's River, 4.VII.1962. United States: SOUTH DAKOTA: 1 pup. (lar. ex.).[1] Clark Lake, 4 mi east of Springfield, 4.IX.1964 (P. Hudson; SUBL); 1♂ (reared), Missouri River, Springfield, 8.VI.1972 (P. Hudson; SUBL). PENNSYLVANIA: 1♂ (reared), 1 pup. and lar. ex., Ithan Creek at junction of Route 320 and Newtown Road, 2.VI.1953, 19. VIII.1953 (S. S. Roback; ANSP); 1 pup. and lar. ex., Perkiomen Creek, Perkiomenville, 17. VIII.1953 (S. S. Roback; ANSP); 1♂ (reared), Tributary E. Branch White Clay Creek, Lamborntown Road 2.12 mi S.W. London Grove, 23.V. 1969 (S. S. Roback; ANSP). NEW YORK: 1♀ (pup. ex.), small stream, S. end of Sterling Lake, Sterling Forest, 18.VI.1970 (S. S. Roback; ANSP); 1♂ (pup. ex.), small stream 1000 ft up Mt. Peter Road from Rt. 210 E. of Warwick, 22.VI.1970 (S. S. Roback; ANSP). MAINE: 1♂, Narraguagus River, 23.VII.1973 (T. M. Mingo).

[1]Reared (in parentheses) indicates that specimen or specimens have associated pupal and larval exuvia unless otherwise stated, i.e., pup. ex. — only pupal exuvium or lar. ex. — only larval exuvium.

Cricotopus (*s.s.*) *vierriensis* Goetghebuer

Cricotopus vierriensis Goetghebuer 1935: 417
Cricotopus (*Cricotopus*) *vierriensis*: Hirvenoja 1973: 239

All of the specimens listed below under "Specimens Examined" agree closely with Hirvenoja's (1973) description of *Cricotopus* (*s.s.*) *vierriensis*.

Distribution — Central and southern Europe, Canary Islands, Israel, and Afghanistan (Hirvenoja 1973). New to the Nearctic region.

Specimens examined — Canada: MANITOBA: 2♂♂ (1 lar. ex.), 1♀ (reared), Calders Dock, Lake Winnipeg, 16.VIII.1971, 18–31.VIII.1971 (M. Roberts and S. Warwick; FWI), 16–23.VII. 1971 (E. Johnson and M. Roberts; FWI); 1♂, 2♀♀ (pup., lar. ex.), Washaw Bay, Lake Winnipeg, 9.VI.1971 (E. Johnson and M. P. McLean; FWI), 28.VII–6.VIII.1971 (R. Deda and J. Ramballi; FWI), 5–10.VIII.1971 (N. Hooper and E. Johnson; FWI). United States: PENNSYLVANIA: 1♂ (pup. ex.), Gulph Creek near Gulph Mills, —.V.1953 (S. S. Roback; ANSP).

Discussion

Two of the species in the *bicinctus*-group, *C. mackenziensis* and *C. bicinctus* are morphologically very similar. Most of the differences between the two species are related to the smaller size of *C. mackenziensis*. Only the pupal stage of *C. mackenziensis* has distinctive additional

characteristics, i.e. robust and black setae on the cephlothorax. The differences in the color pattern of the hypostomium of the larvae of the two species appears to be consistent but it is difficult to use. The presence or absence of frontal tubercles in the adults appears to be a good characteristic for separating the adults in the Fort Simpson area. The presence of frontal tubercles was consistently correlated with small size (as measured by wing length). However, there were several specimens with wing lengths falling within the range for *C. bicinctus* that had a single, partly developed (vestigial?) frontal tubercle on one side of the head.

The larvae of *C. mackenziensis* and *C. bicinctus* apparently live in the same habitat within rivers in the Fort Simpson area (Rosenberg et al. 1977b). Also, the larvae of these two species share a morphological characteristic not found in other species of *Cricotopus*. This is the presence of small spines on the inner margin of the mandible. Until now this character has been considered diagnostic for *C. bicinctus*, as the known larvae of all other species of *Cricotopus* have mandibles with smooth inner margins.

In this paper only the specimens (except two males with a low A.R.) which agreed with Hirvenoja's (1973) description were identified as *C. bicinctus*. The status of a few adult specimens were undetermined, primarily because they were not associated with immature stages. For example, in material from Hayden Brook, New Brunswick, several males were distinctly smaller than most of the specimens of *C. bicinctus* in the collection. They were about the same size as *C. mackenziensis* but lacked frontal tubercles. By contrast, several males in a collection from the Jock River, Ontario, had small conical frontal tubercles (similar to those of female *C. mackenziensis*), but the length of their wings were equal to or greater than the largest *C. bicinctus*. These forms, and others not mentioned here, constituted a small ($< 5\%$) but distinct group of the specimens from any one locality.

The discovery of two sibling species living sympatrically in the Fort Simpson area raises the possibility that what has been called *C. bicinctus* in the Nearctic is actually a species complex. The reported range for *C. bicinctus* is over most of the Nearctic south of the arctic region, i.e. from California and Florida north to the Yukon Territory and Labrador. This is a large geographical range for a single species. Also the variation in adults that has been noted above might be interpreted as support for the species complex concept. However, the status of *C. bicinctus* in the Nearctic cannot be resolved until associated immature stages and adults are available from all parts of its reported geographical range.

Acknowledgments

Data from the Fort Simpson area were obtained as a result of investigations carried out under the Government of Canada Environmental–Social Program, Northern Pipelines, Task Force on Northern Oil Development. I wish to thank Mrs. M. E. Roussel for preparing and measuring specimens; Dr O. A. Sæther for permitting publication of records of *C. vierriensis* from Lake Winnipeg; Dr D. M. Rosenberg, and Dr J. R. Vockeroth for helpful criticism of the manuscript; and Dr S. S. Roback (ANSP), Dr O. A. Sæther (FWI), and Dr J. E. Sublette (SUBL) for the loan of specimens.

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The Morphology, and Some Observations on the Population Genetics of *Anisakis simplex* Larvae (Nematoda: Ascaridata) from Fishes of the North Atlantic

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BEVERLEY-BURTON, M., O. L. NYMAN, AND J. H. C. PIPPY. 1977. The morphology, and some observations on the population genetics of *Anisakis simplex* larvae (Nematoda: Ascaridata) from fishes of the North Atlantic. J. Fish. Res. Board Can. 34: 105–112.

The morphology of larval *Anisakis simplex* in a subsample taken from Atlantic herring, *Clupea harengus harengus*, in the North Sea (and used in previous in vitro culture experiments) is described, with particular reference to the excretory system.

The morphological similarities of these larvae from *C. harengus harengus* and Atlantic salmon, *Salmo salar*, taken in widely separated locations in the North Atlantic suggests that *Anisakis* larvae Type 1 is *A. simplex*. These findings are substantiated by studies on acid phosphatase polymorphism of the larvae and the known geographic ranges of related species. Frequencies of the acid phosphatase phenotypes varied in different sampling areas, suggesting that different populations of larval *A. simplex* occur in fish in the North Atlantic.

BEVERLEY-BURTON, M., O. L. NYMAN, AND J. H. C. PIPPY. 1977. The morphology, and some observations on the population genetics of *Anisakis simplex* larvae (Nematoda: Ascaridata) from fishes of the North Atlantic. J. Fish. Res. Board Can. 34: 105–112.

Les auteurs décrivent la morphologie d'*Anisakis simplex* larvaire provenant d'un sous-échantillon prélevé du hareng de l'Atlantique, *Clupea harengus harengus*, dans la mer du Nord (et utilisé lors d'expériences antérieures de culture in vitro), avec accent sur l'appareil excréteur.

Les ressemblances morphologiques de ces larves provenant de *C. harengus harengus* avec celles provenant du saumon atlantique, *Salmo salar*, capturé à des endroits très différents en Atlantique Nord, donnent à croire que le Type 1 de larves d'*Anisakis* est *A. simplex*. Des études sur le polymorphisme de l'acide phosphatase des larves et la répartition géographique connue d'espèces apparentées corroborent ces observations. La fréquence des phénotypes de l'acide phosphatase varie dans différentes régions d'échantillonnage, ce qui donne à croire qu'il existe différentes populations de *A. simplex* larvaire dans les poissons de l'Atlantique Nord.

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PIPPY and van Banning (1975) studied the morphology of adult nematodes reared in vitro from van Banning's (1971) experiments with larvae taken from Atlantic herring (*Clupea harengus harengus*) taken at station 21 (Fig. 1), and concluded that *Anisakis* Type 1 in fish in the North Sea was *A. simplex* (Rudolphi, 1809, det. Krabbe 1878). Pippy and van Banning (1975) gave a tabulated summary of morphometric data of the larvae used in the rearing experiment but neither these authors nor van Banning (1971) described the morphology of the larvae. This paper presents a description of the larval *A. simplex* from this population which are then compared with larvae from fishes taken in widely separated locations in the North Atlantic. Morphological comparisons were supplemented by studies on an acid phosphatase polymorphism to test the hypothesis that only one species was represented among *Anisakis* larvae Type 1 in the North Atlantic.

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Materials and Methods

Larval *Anisakis* sp. were collected from Atlantic herring (*Clupea harengus harengus*) and Atlantic salmon (*Salmo salar*) from various localities in the North Atlantic (Fig. 1).

FIG. 1. North Atlantic sampling stations of 3rd stage larvae of *Anisakis simplex* from salmon (circles) and herring (squares). See Table 1 for names of stations.

Larvae for morphological studies were either observed alive or fixed in glycerine alcohol (10% glycerine in 70% ethanol) and cleared in lactophenol. Larvae for histological studies were fixed (48 h) in alcoholic Bouin's solution and washed (24 h) in 70% ethanol. Specimens were then bisected, dehydrated in ethanol (70, 90, 100%), and cleared in chloroform. Specimen lengths of 1.5 mm were embedded in Paraplast. Sections were cut at 10 μm and stained with Ehrlich's hematoxylin and eosin.

Starch-gel electrophorectic analyses, using frozen larvae, were made in a discontinuous buffer system as described by Ashton and Bradon (1961). The gel, poured to a thickness of 6 mm, was cooled in a plexiglass mold 24.5 × 14.5 cm. Each of 861 larvae was thawed and crushed with a scalpel on a labeled microscope slide with 0.01 mℓ of the same buffer mixture as that used in gel preparation. The diffusate was absorbed into a 2.5 × 6-mm piece of chromatographic paper (Watman no. 1). Preparations for analysis were slotted (2.5 mm apart) into a continuous vertical cut parallel to, and 40 mm from one of the long edges of the starch gel. Electrophoresis was conducted for 120 min at 400 V with water cooling provided by an apparatus similar to that used by Nyman (1965). For staining, each gel was sliced horizontally using a thin taut wire between the upper and lower mold frames so that only the lower half was used. The gel was incubated in a sodium acetate buffer pH 5.0 (Burstone 1962) for 30 min which was replaced by a staining solution (100 mℓ of fresh sodium acetate buffer, 100 mg Fast Blue RR, and 100 mg alpha naphythyl acid phosphate). The staining vessel was covered with plastic film and incubated 6–8 h at 37°C. After staining, the distance of each band from the origin was measured with the aid of dividers and recorded on graph paper. The gels were preserved in glycerine.

Results

DESCRIPTION OF LARVAE

Based primarily on the larvae used by Pippy and van Banning (1975) from Station 21 together with larvae from other stations.

Larvae (Fig. 2) 19.69 (16.11–22.49 mm) long, 0.49 (0.42–0.56 mm) diam. Tail rounded with retractable terminal spine, 0.029 mm long (Fig. 2B); cuticle, 10–15 μm thick, usually striated near head and posterior extremity (Fig. 2B and C), but some worms striated along entire body length. Mouth triangular with trilobed dorsal lip and bilobed ventrolateral lips each with single indistinct papilla. Anteroventrally projecting boring tooth located ventral to the mouth (Fig. 2D). Fine transverse striations visible at extreme anterior end of esophagus; area immediately posterior granular in appearance (Fig. 2C). Dorsal esophageal gland extends just anterior to nerve ring; 2 ventrolateral glands small, located near posterior end of esophagus (Fig. 2A). Ventral side of ventriculus slightly longer than dorsal side; intestinal–rectal valve short, lined externally by 4 epithelial cells. Three rectal glands: 2 dorsal and 1 ventral. Lateral cervical papillae located about 0.3 mm behind nerve ring (Fig. 2A).

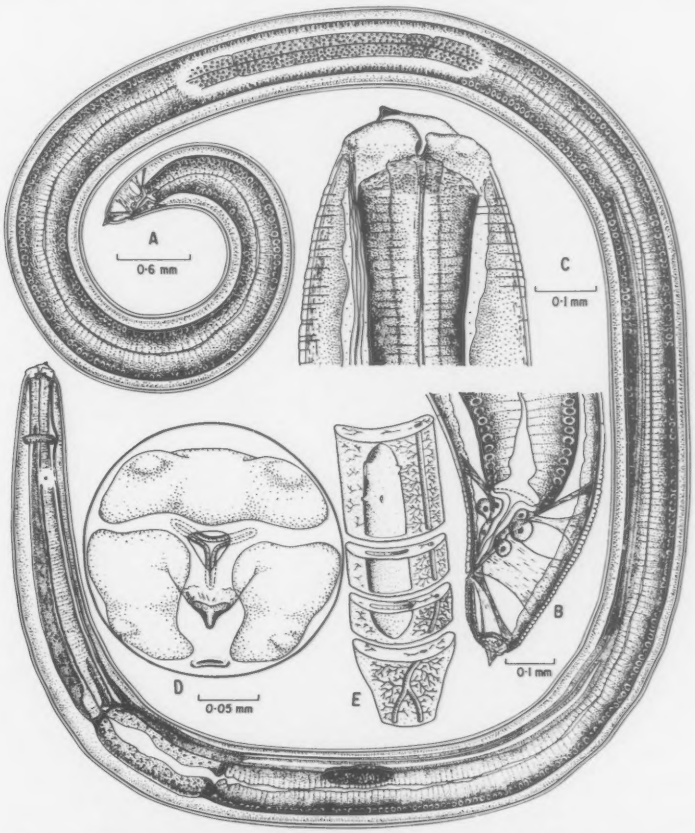


FIG. 2. Morphology of 3rd stage larva of *A. simplex*: A, entire worm; B, tail; C, head; D, en face view; E, structure of the excretory cell (diagrammatic). Figures A–D were drawn with the aid of a camera lucida.

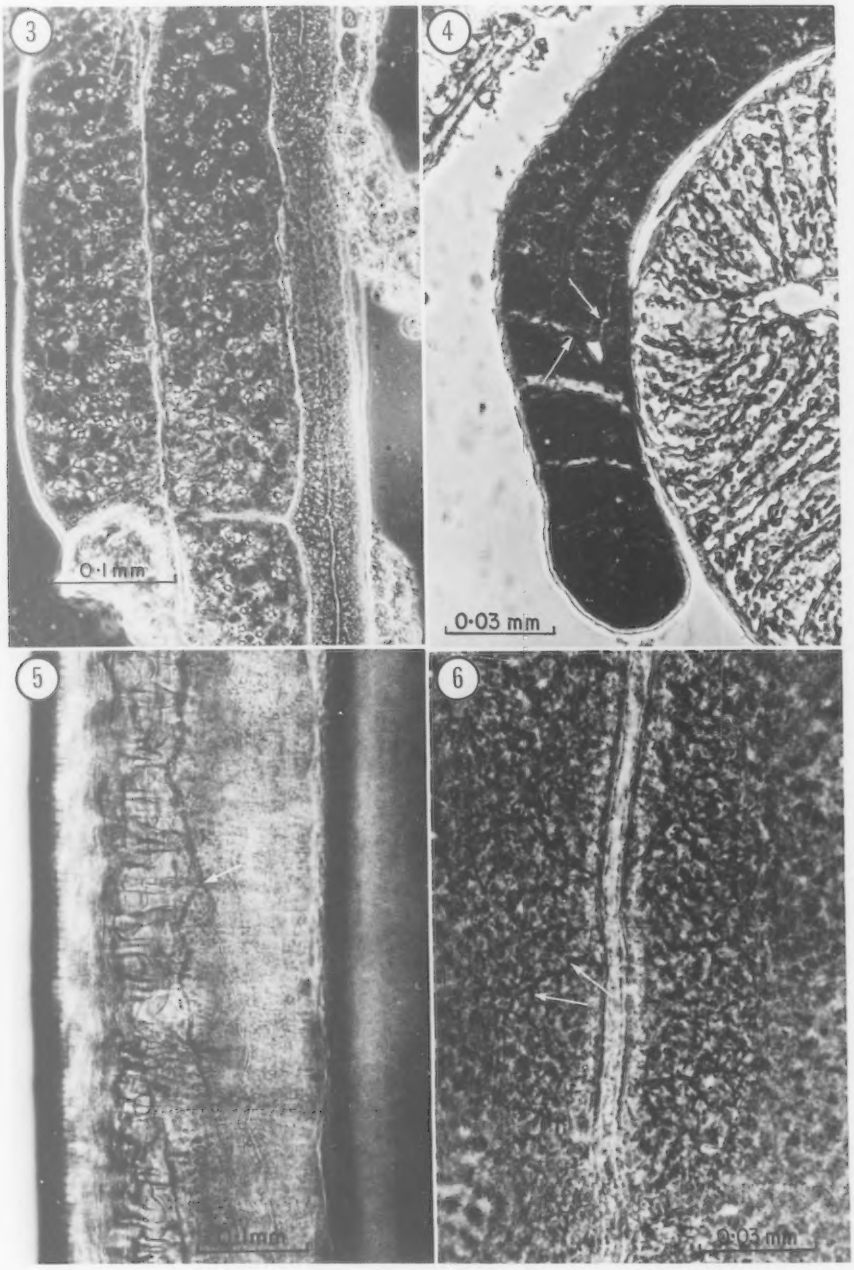
Excretory pore between ventrolateral lips with single excretory duct passing up from excretory cell. Latter extends posteriad from just anterior to ventriculus to a level 6.78–8.40 mm from anterior end of body. Except at extremities, where rounded in cross section, excretory cell elongate to oval in cross section and contiguous to hypodermal cells (Fig. 3), alimentary canal (Fig. 4), and occasionally, somatic musculature. Two small ducts located in narrow posterior portion of cell merge to form single excretory duct (Fig. 2A and E, Fig. 5) with numerous fine tubules ramifying throughout cell cytoplasm (Fig. 2E and 6). Nucleus large, strongly acidophilic, extends from vicinity of posterior end of ventriculus to bifurcation of excretory duct (Fig. 2A). Anterior end of nucleus with 4 or 5 extensions reaching ventral wall of excretory cell; 2 most anterior extensions largest (Fig. 2E).

Length of hypodermal cells (Fig. 7) 0.065–3.68 mm long (mean 1.10 mm). Genital primordium (from sectioned material) consisting of a group of cells lying in pseudocoel 1–2 mm posterior to ventriculus and surrounded by intestine, hypodermal cells, somatic musculature, and excretory cell (Fig. 8).

COMPARISONS OF LARVAE FROM DIFFERENT AREAS

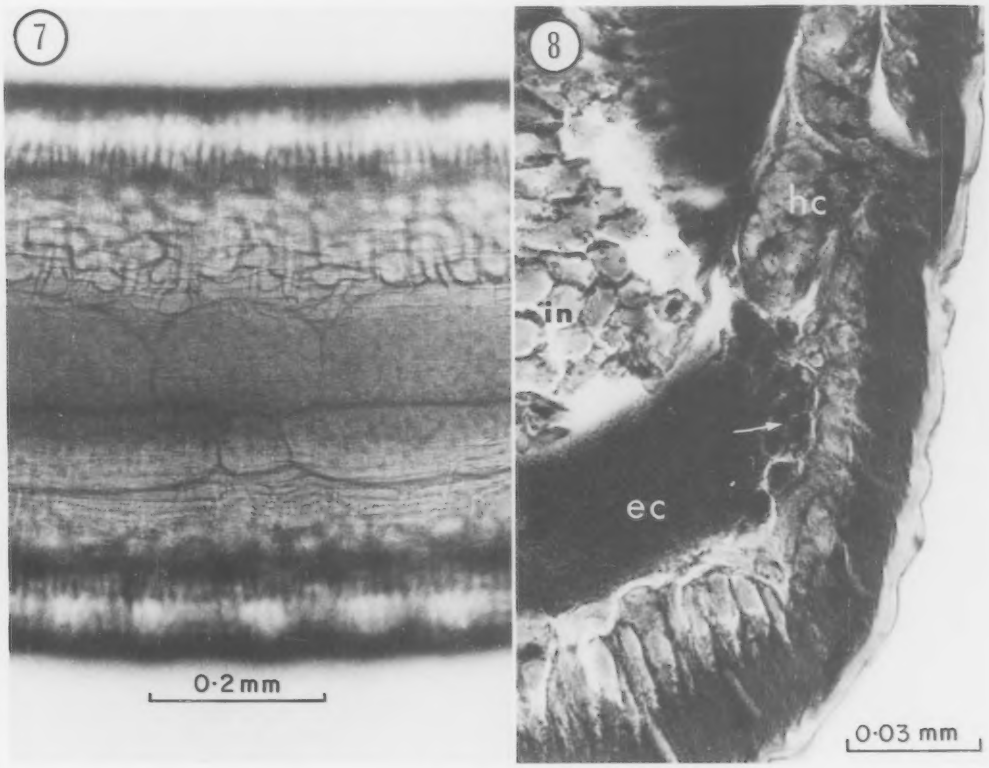
Morphology — Larvae collected off West Greenland (Station 1), Canada (Stations 2–15), and in the eastern Atlantic (Stations 16–21) were morphologically similar to one another and to the specimens just described.

Acid phosphatase phenotypes — A total of 5 different bands of acid phosphatase isozymes, either alone or in pairs, were detected among the



FIGURES 3–6.

FIG. 3. Anterior region of excretory cell (right) duct. Note proximity of hypodermal cells to excretory cell. Dissected specimen. FIG. 4. Cross section of anterior end of excretory cell showing its proximity to alimentary canal and two fine tubules (arrows) from the excretory duct. FIG. 5. Confluence of excretory cell ducts (arrow). Excretory cell in situ. FIG. 6. Branching tubules (arrows) in cytoplasm of excretory cell. Phase contrast — dissected specimen.



FIGURES 7 AND 8.

FIG. 7. Portions of 6 hypodermal cells (in situ) showing variation in size. FIG. 8. Cross section showing genital primordium (arrow). Note excretory cell (ec), hypodermal cell (hc), body wall, and intestine (in).

861 larvae examined. These bands were labeled A_1, A, B, C, and D and were assumed to be related to the presence of 5 codominant alleles: P^{A1}, P^{A}, P^{B}, P^{C}, and P^{D}. They were labeled according to their decreasing mobility in the starch gel (Fig. 9). Phenotypes with a single band were considered to be homozygous whereas those with two bands were considered to be heterozygous.

Zymograms of larvae from different samples differed from each other only with respect to frequency of occurrence of different phenotypes. Phenotypic frequencies obtained from samples of larvae from salmon taken off northern Newfoundland (Station 4), in the Chaleur Bay and Miramichi areas (Stations 11 and 12, respectively), in Scotland (Station 20), and from herring taken off Nova Scotia (Station 15) were compared with those expected on the basis of the Hardy–Weinberg–Castle equilibrium (Table 1). In each case, chi-square analyses indicated good agreement with

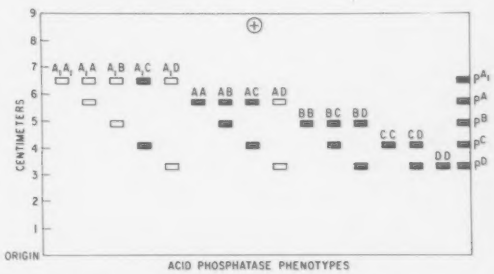


FIG. 9. Observed (dark bands) and hypothesized (light bands) acid phosphatase band patterns from 3rd stage larval *Anisakis simplex*.

the hypothesis that the system was in genetic equilibrium with respect to the P^{B} and P^{C} alleles. The A_1, A, and D bands occurred too infrequently for similar statistical analyses. Thus, the available data on samples from Stations 4, 11, 12, 20, and 15 were consistent with the hypothesis that these

TABLE 1. Observed (OBS) and expected (EXP) frequencies of acid phosphatase phenotypes of 3rd stage larvae of *Anisakis simplex* and chi-square analyses on some of the more prevalent phenotypes. Expected phenotypic frequencies were determined by assuming an H–W–C equilibrium in the samples and allele frequencies are given with their respective standard errors; X indicates all alleles other than the one presented first in each chi-square analysis; NS = not statistically significant at the 95% level.

Station	Packs Hr (3)		St. Anthony (4)		Chaleur B. (11)		Miramichi B. (12)		W. Fundy (13)	
Number larvae	158		135		79		32		201	
Phenotype	OBS	EXP	OBS	EXP	OBS	EXP	OBS	EXP	OBS	EXP
Allele frequencies										
fP^{A_1}	0.000		0.000		0.000		0.000		0.003 ± .003	
fP^{A}	0.051 ± .012		0.048 ± .013		0.057 ± .018		0.047 ± .026		0.052 ± .011	
fP^{B}	0.434 ± .028		0.474 ± .030		0.373 ± .039		0.438 ± .062		0.475 ± .025	
fP^{C}	0.513 ± .028		0.474 ± .030		0.563 ± .040		0.516 ± .062		0.468 ± .025	
fP^{D}	0.003 ± .003		0.004 ± .004		0.006 ± .006		0.000		0.003 ± .003	
χ^2 Analyses										
BB	40	29.70	36	30.34	13	11.02	9	6.13	67	45.37
BX	57	77.60	56	67.32	33	36.97	10	15.75	57	100.25
XX	61	50.79	43	37.34	33	31.02	13	10.13	77	55.37
χ^2	10.34		3.33^{NS}		0.60^{NS}		3.23^{NS}		36.13	
CC	52	41.53	36	30.34	26	25.07	11	8.51	63	43.96
CX	58	78.95	56	67.32	37	38.87	11	15.75	62	100.08
XX	48	37.53	43	37.34	16	15.07	10	7.51	76	56.96
χ^2	10.34		3.33^{NS}		0.07^{NS}		2.25^{NS}		27.97	

Station	E Nova Scotia (15)		Ireland (18)		England (19)		Scotland (20)	
Number larvae	90		29		98		39	
Phenotype	OBS	EXP	OBS	EXP	OBS	EXP	OBS	EXP
Allele frequencies								
fP^{A_1}	0.011 ± .008		0.000		0.000		0.000	
fP^{A}	0.050 ± .016		0.017 ± .017		0.082 ± .020		0.077 ± .030	
fP^{B}	0.439 ± .037		0.293 ± .060		0.383 ± .035		0.500 ± .057	
fP^{C}	0.500 ± .037		0.596 ± .065		0.520 ± .036		0.423 ± .056	
fP^{D}	0.000		0.121 ± .043		0.015 ± .009		0.000	
χ^2 Analyses								
BB	20	17.34	8	2.49	21	14.35	12	9.75
BX	39	44.33	1	12.02	33	46.30	15	19.50
XX	31	28.34	20	14.49	44	37.35	12	9.75
χ^2	0.96^{NS}		21.01		7.19		1.45^{NS}	
CC	23	22.50	15	9.39	35	26.54	10	6.98
CX	44	45.00	3	14.22	32	48.92	13	19.04
XX	23	22.50	11	5.39	31	22.54	16	12.98
χ^2	0.01^{NS}		15.72		10.71		3.01^{NS}	

Station	(11+12)		(4+11+12)		(4+11+12+15)		All larvae	
Number larvae	111		246		336		861	
Phenotype	OBS	EXP	OBS	EXP	OBS	EXP	OBS	EXP
Allele frequencies								
fP^{A_1}	0.000		0.000		0.003 ± .002		0.002 ± .001	
fP^{A}	0.054 ± .023		0.051 ± .010		0.051 ± .009		0.055 ± .005	
fP^{B}	0.392 ± .049		0.437 ± .022		0.506 ± .019		0.437 ± .012	
fP^{C}	0.550 ± .050		0.508 ± .023		0.506 ± .019		0.498 ± .012	
fP^{D}	0.005		0.004 ± .003		0.003 ± .002		0.008 ± .002	
χ^2 Analyses								
BB	22	17.05	58	46.98	78	64.31	226	164.64
BX	43	52.91	99	121.05	138	165.38	301	423.73
XX	46	41.05	89	77.98	120	106.31	334	272.64
χ^2	3.32^{NS}		7.61		8.71		71.34	
CC	37	33.52	73	63.52	96	84.00	271	213.75
CX	48	54.96	104	122.97	144	168.00	316	430.50
XX	26	22.52	69	59.52	96	84.00	274	216.75
χ^2	1.42^{NS}		5.40		6.44		60.11	

larvae were drawn from single interbreeding populations. However, these analyses did not indicate that the samples from the above stations were originally all derived from the same interbreeding population. Significant deviations from expected phenotypic frequencies were observed when samples from Station 4, 11, and 12 (larvae from Canadian salmon only) were combined, when these were combined with the sample from herring (Station 15) in Canada, and when all samples collected were combined (Table 1). These analyses indicated the presence of more than one population among larvae in the North Atlantic region, and that salmon and herring in different areas may acquire larvae from different populations.

Evidence for the presence of more than one population in the North Atlantic was also observed in single samples; significant deviations from expected phenotypic frequencies were observed in samples from Labrador (Station 3), the Bay of Fundy (Station 13), Ireland (Station 18), and England (Station 19) (Table 1).

There was no evidence of significant differences between different samples with respect to the frequency of P^{A_1}, P^{A}, P^{C}, and P^{D} alleles (Fig. 10). However, the frequency of the P^{B} allele in the sample from Ireland (Station 18) was significantly lower than the frequency in the sample from the Bay of Fundy (Station 13). This indicated that at least one population of the parasite had a relatively low frequency of occurrence of the P^{B} allele.

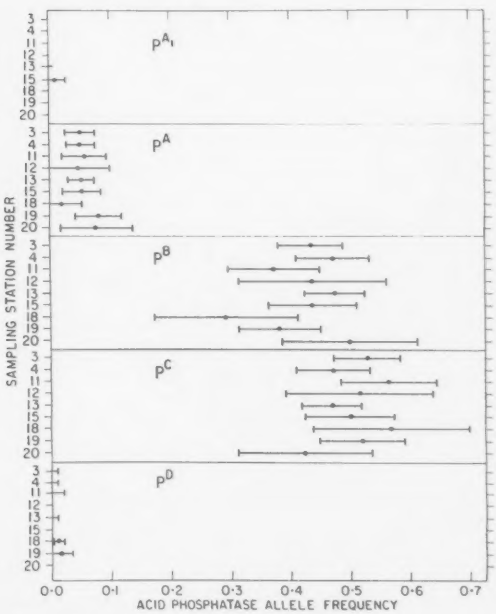


FIG. 10. 95% confidence limits of the frequencies of occurrence of 5 acid phosphatase alleles in 3rd stage larvae from 9 sampling stations in the North Atlantic.

Discussion

Berland (1961) did not fully redescribe *Anisakis* Type I larvae but referred to descriptions by Grainger (1959) and Punt (1941). However, Berland's drawing of the larvae did not fully agree either with the description and figure of the cephalic structures of *Anisakis* sp. larvae given by Grainger (1959) or with the description of the tail given by Punt (1941). Another difficulty in referring to Punt's description as being representative of *Anisakis* Type I larvae is that a specimen with an anteriorly directed caecum was mentioned. Undoubtedly, this specimen was not *Anisakis* sp. Berland's illustrations of *Anisakis* Type I larvae conform in all other respects to Punt's description of *Anisakis* sp. With the above reservations, the specimens examined here agree with the description of Berland's *Anisakis* Type I larvae.

Previous descriptions of the excretory system of *Anisakis* Type I larvae are incomplete (Jagerskiöld 1894; Wülker 1930; Punt 1941; Grainger 1959; Berland 1961; Oshima 1972). The excretory cell of the present larvae differs in several respects from that described for adult *A. simplex* by Mueller (1927). Mueller did not mention either that the extensions of the nucleus to the wall of the cell are limited to the anterior part of the nucleus, or the bifurcation of the excretory duct in the narrow posterior portion of the cell. These differences may be related to the ontogeny of the species.

The morphological similarities of the larvae examined from both sides of the North Atlantic to larval *A. simplex* from the North Sea suggests that Type I larvae throughout the North Atlantic represent a single species. In contrast, the data on the population genetics of the larvae in some samples suggest that either more than one species is present or that more than one population of the same species is present. The possibility that larval *A. physeteris* is involved may be discounted on the basis that larval *A. physeteris* (= *Anisakis* Type II larvae) are likely morphologically different from Type I larvae (Oshima 1972). However, larval *A. simplex* and *A. typica* may very well be morphologically and morphometrically similar, and indistinguishable by the

methodology used here. This difficulty may be clarified by a comparison of the geographic ranges of *A. simplex* and *A. typica* with the observed genetic heterogeneity among the larvae examined.

Davey (1971) noted that the geographic distribution of adult *A. typica* and *A. simplex* were contiguous and that *A. typica* filled a geographic gap in the otherwise cosmopolitan distribution of *A. simplex*. Records of *A. typica* were from warmer temperate and tropical waters between 40°N and 36°S. All of the larvae examined in this study were taken from fish collected north of 40°N and there has been no published evidence to indicate that Atlantic salmon from which the larvae were taken migrate south of this latitude. Tagging experiments by Pratt et al. (1974) indicated that few salmon originating in Labrador migrate south of 50°N. Thus, the sample from Labrador (Station 3) is unique in that practically all the larvae must have been acquired in waters north of 50°N and the probability that larval *A. typica* was present in the sample is therefore extremely low. Nevertheless, there was poor agreement with observed and expected acid phosphatase phenotypic frequencies in this sample, indicating the presence of more than one population of the nematode (Table 1).

In this same context, the sample of larvae from Atlantic herring collected just east of Nova Scotia (Station 15) warrants consideration. Data on this stock of herring (Parsons 1973) indicated that it ranges as far south as 40°N. Thus, if the larvae of *A. typica* extend this far north, they would more likely be represented in this particular sample than in any of the samples from salmon. However, the observed phenotypic frequencies of acid phosphatase isozymes at Station 15 agreed extremely well with expected frequencies (Table 1). This suggests that a single population of larvae was present in the Nova Scotian sample and supports the suggestion that practically all the specimens belong to the same species. Data from this sample are inconsistent with the suggestion that more than one species is represented in samples taken further north. The discrepancies between observed and expected frequencies of acid phosphatase phenotypes in many of the samples must, therefore, be attributable to the existence of more than one population of *A. simplex* in the North Atlantic rather than a mixture of larval *A. simplex* and *A. typica*.

Only one species is represented among *Anisakis* Type I larvae in herring and salmon in the North Atlantic, namely, *A. simplex*.

Acknowledgments

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Instar Succession, Vertical Distribution, and Interspecific Competition Among Four Species of *Chaoborus*[1]

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CARTER, J. C. H., AND J. K. KWIK. 1977. Instar succession, vertical distribution, and interspecific competition among four species of *Chaoborus*. J. Fish. Res. Board Can. 34: 113–118.

Of four highly oligotrophic lakes of the Matamek River System, Quebec, Lac à la Croix contains *Chaoborus punctipennis* and *C. trivittatus*, Lac Gallienne *C. americanus*, Lac Randin *C. americanus* and *C. trivittatus*, and Lac Méchant *C. flavicans* and *C. trivittatus*. The instars are all planktonic except instars I–III of *C. trivittatus* in Lac à la Croix. All species have a 1-yr life cycle except *C. americanus* in Lac Gallienne which has a 2-yr cycle. The cycles of *C. trivittatus* in lacs Randin and Méchant are out of phase by 1 mo or more with those of *C. americanus* and *C. flavicans*, respectively. Seasonal increases in length occurred in most instars except those of *C. punctipennis* in Lac à la Croix. Both species in Lac à la Croix made fairly extensive diurnal migrations but in lacs Gallienne and Méchant all species remained near the surface. Intense competition for food is considered the most likely cause of seasonal or spatial separation of instars of similar size in all lakes.

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Parmi quatre lacs oligotrophes du réseau de la rivière Matamek, Québec, le lac à la Croix contient *Chaoborus punctipennis* et *C. trivittatus*, le lac Gallienne *C. americanus*, le lac Randin *C. americanus* et *C. trivittatus*, et le lac Méchant *C. flavicans* et *C. trivittatus*. Les larves sont toutes planctoniques, à l'exception des larves I–III de *C. trivittatus* dans le lac à la Croix. Toutes les espèces ont un cycle biologique d'1 a, sauf *C. americanus* dans le lac Gallienne, qui a un cycle de 2 a. Les cycles de *C. trivittatus* dans les lacs Randin et Méchant sont décalés d'un mois ou plus par rapport à ceux de *C. americanus* et *C. flavicans* respectivement. Il y a accroissements saisonniers de longueur dans la plupart des larves, sauf celles de *C. punctipennis* dans le lac à la Croix. Les deux espèces du lac à la Croix entreprennent des migrations nycthémérales assez prononcées, mais dans les lacs Gallienne et Méchant, toutes les espèces demeurent près de la surface. Une intense compétition pour la nourriture est considérée comme étant la cause la plus probable de la séparation saisonnière ou spatiale de larves de même taille dans tous les lacs.

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THE frequent cooccurrence of *Chaoborus* larvae has always posed a problem since it suggests that Hardin's (1960) competitive exclusion principle may not have universal application. Stahl (1966a) supposed that, while coexisting species probably had identical ecological requirements, competition was eliminated through a superabundance of resources. Recent findings by Fedorenko (1975) that the diet of planktonic *Chaoborus* in Eunice Lake, British Columbia, is governed more by spatial distribution of predator and prey than by prey size relative to predator size suggests that interspecific competition may well arise if two or more species occupy the same strata of a lake.

Because of their vulnerability to predation by fish, larval *Chaoborus* are seldom permanently planktonic, preferring either the benthos or deepest hypolimnetic waters, at least during the day (cf. Berg 1937; Stahl 1966b; Roth 1968). However, in fishless lakes (cf. Fedorenko and Swift 1972) or lakes with low fish stocks they are often found only in the water column. There the smaller instars are easily collected making it possible to establish relationships between coexisting species in time and place.

The present paper examines population densities, life cycles, size relationships, vertical distributions, and diurnal migrations as they relate to interspecific competition in four *Chaoborus* species

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in four highly oligotrophic lakes, two with fish and two without.

Description of Study Area

The Matamek River is situated on the north shore of the Gulf of St. Lawrence, about 30 km east of Sept-Iles, Quebec (Fig. 1). The northern two thirds of the river system has remained without natural fish populations following the last glaciation since the steep escarpment between the Champlain Plain and the Labrador Plateau has provided an insuperable barrier to recolonization. Pope et al. (1973) showed that, of 26 lakes in the system, those with fish contained both different species combinations and lower numbers of *Chaoborus* than those without fish.

Of the four lakes in this study Lac Randin (area 43 ha, maximum depth 67 m) is fishless and contains *Chaoborus americanus* and *C. trivittatus*; Lac Méchant (area 318 ha, maximum depth 98 m) is also fishless and contains *C. flavicans*, *C. trivittatus*, and small numbers of *C. americanus*; Lac Gallienne (area 40 ha, maximum depth 43 m) was formerly fishless until stocked with Atlantic salmon, *Salmo salar*, in 1970 and contains *C. americanus* and occasional individuals of an unidentified species of the subgenus *Schadanophasma* (this was present in much greater numbers in 1970 but has apparently been almost eliminated by the salmon); Lac à la Croix (area 277 ha, maximum depth 73 m) has low numbers of five species of fish (brook trout, *Salvelinus fontinalis*, Arctic char, *S. alpinus*, rainbow smelt, *Osmerus mordax*, ninespine stickleback, *Pungitius pungitius*, and threespine stickleback, *Gasterosteus aculeatus*) and contains *C. punctipennis* and *C. trivittatus*.

Ice usually leaves the lakes in late May or early June. All four lakes stratify during the summer with thermoclines at 5–8 m. They normally freeze over by mid-November.

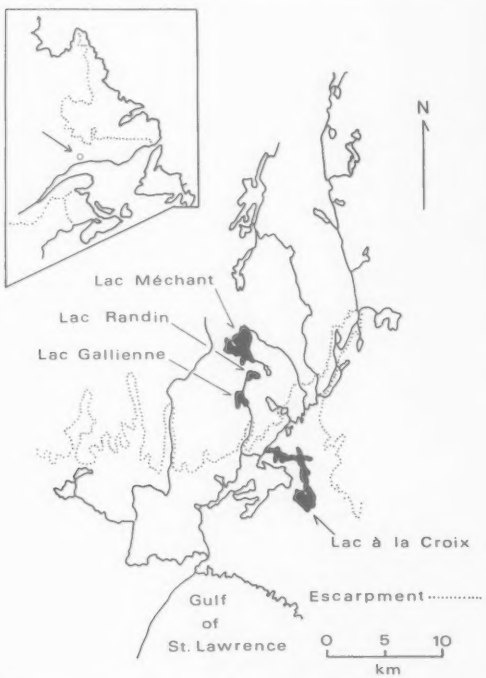

FIG. 1. The Matamek River System, Quebec, showing the four lakes of the study and the escarpment separating the Champlain Plain (south) from the Labrador Plateau (north).

Materials and Methods

A conical plankton net of 0.5 m mouth diam and no. 10 mesh (apertures 0.158 mm) was used. During 1974 four daytime vertical hauls from bottom to surface were taken at each of three stations in the northern basin of Lac à la Croix (maximum depth 22 m), four stations in Lac Gallienne and one station in Lac Randin on 13 occasions from mid-June to early October. In 1975 one station in the western basin of Lac Méchant (maximum depth 50 m) was similarly sampled on 13 occasions from early June to early November. Since the lakes are accessible only by float plane, helicopter, or extremely arduous hiking, intervals between sampling dates were necessarily unequal. Vertical hauls in conjunction with benthic sampling were also carried out once each in March 1975 through the ice and in June 1975 at one station in both lacs Gallienne and à la Croix. These were the only benthic samples taken during the study, each consisting of three hauls with a 22.5 × 22.5-cm Ekman Grab. All samples from each lake on each sampling date were combined. Vertical distributions and extent of diurnal migrations were determined by fitting a closing device on the conical net and sampling individual depth strata; one series of depth hauls at intervals over about 20 h was made at the deepest station in each of lacs à la Croix, Gallienne, and Méchant. Temperatures of the upper waters were taken during each sampling visit using a thermistor.

Identifications to species of later instars were made following Sæther (1972). Instar determinations were worked out in accordance with length of head capsule which is relatively constant for each instar (Fig. 2) and independent of total body length. Since consider-

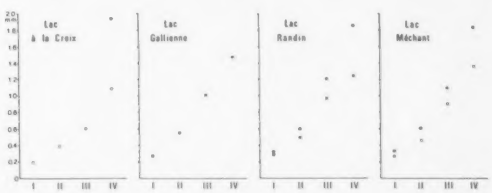

FIG. 2. Mean head capsule lengths of *Chaoborus trivittatus* (●), *C. punctipennis* (○), *C. americanus* (■), and *C. flavicans* (□) in the four lakes. A minimum of 30 animals in each instar were measured. SD < 0.08 mm.

able differences in head capsule length usually occurred between equivalent instars of coexisting species it was a simple matter to identify a homogeneous group of younger instars even though this occasionally entailed working backwards in time from the older stages of the same species. All specimens from each combined sample were counted and at least 30 members of each instar when available were measured for total body length. Only an occasional pupa of any species was found in the samples and they are therefore not included in this study.

Results and Discussion

Population Densities

At all times the numbers per square meter were considerably lower than is generally found in more eutrophic situations (cf. Berg 1937; Roth 1968). Maximum densities recorded in numbers per square meter for *C. punctipennis* were 770 (instar III); for *C. trivittatus* 186 (Lac Méchant, instar I), 41 (Lac à la Croix, instar IV), and 17 (Lac Randin, instar III); for *C. americanus* 1627 (Lac Gallienne, instar II), and 644 (Lac Randin, instar II); and for *C. flavicans* 684 (instar II).

Densities of *C. trivittatus* were particularly low, the populations being smaller than those of its congener in lacs à la Croix, Randin, and Méchant. This may be simply because of its larger size and greater nutritional requirements, or it is possible that some individuals of *C. trivittatus* were benthic as was the case in Lac à la Croix in March; instars I, II, and III of this species were never found in the plankton in Lac à la Croix. Although only instar III was found in the limited number of benthic samples, the most likely hypothesis is that the younger instars were permanently benthic, a curious reversal since previous studies have indicated earlier instars always tend to be more planktonic than older ones (Eggleton 1932; Berg 1937; Wood 1956; Teraguchi and Northcote 1966). An alternative explanation, that the instar IV found in 1974 were the result of a late crop of larvae produced during 1973 which failed to pupate, emerge, and reproduce during 1974, might also be considered.

Chaoborus punctipennis also was partially benthic during March and June 1975. In Lac Gallienne no specimens were found in the benthic hauls taken at these times.

Life Cycles

In Lac à la Croix *C. punctipennis* overwintered mainly as instar IV with a small fraction as instar III (Fig. 3). Emergence was quite late, instar I appearing only in mid-July with a pulse median on August 6 (Table 1). By early October a slight majority was still at instar III.

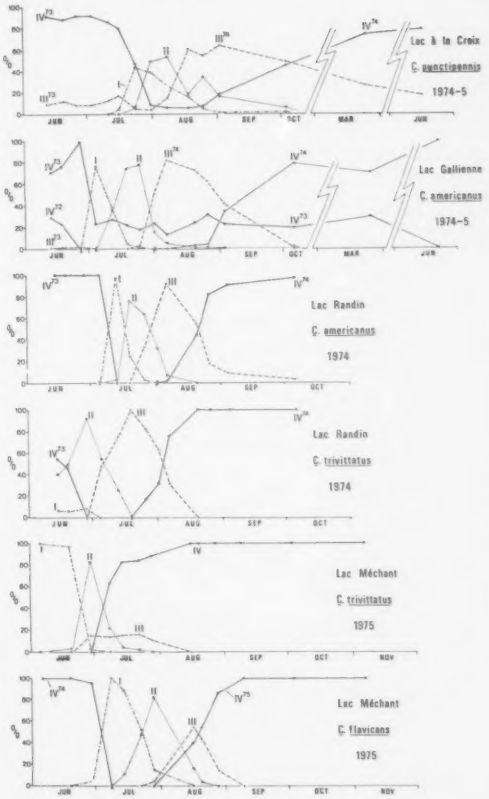

FIG. 3. Larval instar succession of *Chaoborus* species in the four lakes of the Matamek River System.

TABLE 1. Dates of pulse medians[a] of planktonic instars I–III for each major *Chaoborus* species in the four lakes. Asterisk indicates estimated date where pulse is incomplete.

	I	II	III
	Lac à la Croix – 1974		
C. punctipennis	Aug. 6	Aug. 21	Sept. 10*
	Lac Gallienne		
C. americanus	July 10	July 22	Aug. 24
	Lac Randin		
C. americanus	July 18	July 22	Aug. 6
C. trivittatus	June 15*	June 24*	July 12
	Lac Méchant		
C. trivittatus	June 7*	June 29	July 8
C. flavicans	July 24	July 30	Aug. 17

[a]Represents the point when half the members of a cohort have passed through a particular instar, assuming that mortality rate and instar development time are roughly constant over the duration of the pulse.

In Lac Gallienne *C. americanus* is postulated to have a 2-yr life cycle, the fourth instar surviving a second summer and having a total duration of about 21 mo. Larvae that had molted to instar IV in 1972 (IV^{72}) were larger and much darker in color than those that had molted to instar IV in 1973 (IV^{73}); the latter in turn grew larger and darker through the summer to eventually show the same distinguishing features in relation to IV^{74}. Fedorenko and Swift (1972) found that *C. trivittatus* in Eunice Lake had a 2-yr life cycle with the same morphological differences between year-classes. Instar IV^{72} had all emerged by late June 1974 with instar I appearing shortly after. By early October almost all of the new generation had reached instar IV^{74} while about 20% of the total population were entering their second winter as instar IV^{73}; by late June 1975 these latter had presumably emerged although a new generation had not yet appeared. Examination of samples collected from Lac Gallienne by Pope in July and August of 1969 and 1970 indicates that at that time *C. americanus* had a 1-yr life cycle. The virtual elimination of the larger *Schadanophasma* species may have opened a niche for a larger instar IV of *C. americanus* which it is now able to develop by means of a 2-yr life cycle.

In contrast, the life cycle of *C. americanus* in Lac Randin was 1 yr only. In addition, there is a suggestion that here the earlier part of the larval cycle also was somewhat accelerated: the duration from the pulse median of instar I to that of instar III was only 19 days while in Lac Gallienne the same process required 45 days (Table 1). In Lac Randin *C. trivittatus* preceded *C. americanus* by about 1 mo: instars I and II were already present by mid-June while the pulse median of instar III fell 25 days earlier than that of *C. americanus*. A situation similar to that in Lac Randin prevailed in Lac Méchant: *C. trivittatus* preceded *C. flavicans* by 1 mo or more. Apparently *C. trivittatus* in Lac Méchant must have pupated and emerged as soon as the ice melted since no instar IV were present on June 5, the first sampling date.

Instar Lengths

In the majority of cases instars showed a seasonal increase in mean body length (Fig. 4). In Lac Gallienne in October 1974, *C. americanus* 1st-yr fourth instars (IV^{74}) had attained about 80% of the body length reached by 2nd-yr fourth instars (IV^{73}). Comparison of lengths of old fourth instars from the earliest spring sampling dates with lengths of new fourth instars from the final autumn sampling in all lakes suggests that

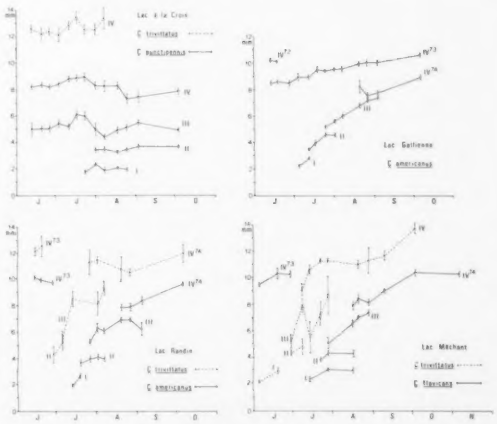

FIG. 4. Seasonal changes in instar lengths in the four lakes of the Matamek River System. Means and 95% confidence limits are given.

seasonal growth ceases in October, doubtless a result of low temperatures and decreased food.

An apparent exception to the trend toward seasonal growth is that of *C. punctipennis* in Lac à la Croix where the four instars maintained relatively constant mean lengths through the season. The smallest *Chaoborus* species in the lakes of the system is *C. punctipennis* and it is found in all lakes containing two or more species of fish. This suggests that there may be some selection against rapid growth to ultimately reduce final size, particularly of instar IV, as a protection against predation. Examination of fish stomachs in 1970 indicated a definite preference for *C. trivittatus* (Pope et al. 1973).

Interspecific competition may be considerably lessened if instars of similar size do not cooccur in the plankton since larger instars invariably have larger head capsules (see Fig. 2) and greater mouth gape which is a major determinant in the size of prey ingested (Swift and Fedorenko 1975). Figure 4 indicates that in lacs Randin and Méchant, because life cycles of coexisting species are so out of phase, instars of similar size never cooccur. In Lac à la Croix timing of the life cycle of *C. trivittatus* is unknown, but the fact that the three earliest instars are probably benthic is suggestive since instars II and III would be roughly similar in size to instars III and IV of *C. punctipennis* both of which are always present in the plankton.

In Lac Méchant *C. americanus* occurs only sporadically although a small lake nearby contains relatively large numbers (Lake LO-1; see Pope et al. 1973). Where they occur in the four lakes *C. americanus* and *C. flavicans* have similar

characteristics: their life cycles are approximately in phase (Fig. 3) and their instar lengths are equivalent. It is possible that their niches overlap to such an extent that *C. americanus* is unable to obtain a permanent foothold in Lac Méchant so long as *C. flavicans* is established there.

Vertical Distribution and Diurnal Migration

The four species showed somewhat different depth preferences. In Lac à la Croix both *C. trivittatus* (instar IV) and *C. punctipennis* (instar IV) reached the surface only at night, descending to deeper strata after midnight (Fig. 5). This migration to darker waters during daylight is probably a defence against planktivorous fish particularly since *C. trivittatus*, the larger and more vulnerable species, always remained somewhat deeper than *C. punctipennis*. Fedorenko and Swift (1972) found a similar migratory tendency in *C. trivittatus* in Eunice Lake although there were no fish.

In Lac Gallienne *C. americanus* (instars, I, II, and IV[73]) showed little inclination to migrate diurnally (Fig. 6). The majority were found in the upper 3 m at almost all times although a small fraction of each instar underwent reverse migration and descended to the deepest waters at night. The tendency to remain in the uppermost waters during daylight is possibly the reason why *C. americanus* is found only in the fishless lakes of the Matamek River System.

In Lac Méchant instars III and IV of *C. flavicans* likewise were concentrated in the upper waters although Instar IV was found only below 5 m at midday (Fig. 7). Other workers have found that the older instars of *C. flavicans* migrate to either the deeper waters of the sediments before daylight (Berg 1937; Stahl 1966b; Roth 1968; Golspink and Scott 1971). Instar IV of *C. trivittatus* in Lac Méchant behaved quite differently than it did in Lac à la Croix, remaining for the most part in the 5–10-m stratum at all times.

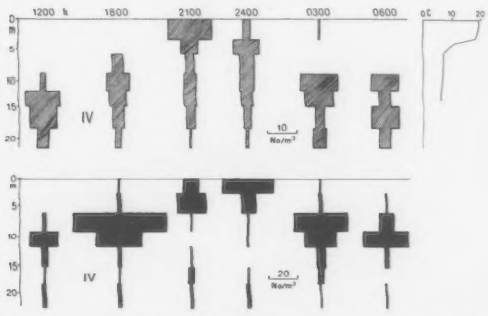

FIG. 5. Diurnal vertical migration of instar IV of *C. trivittatus* (*above*, hatched) and of instar IV of *C. punctipennis* (*below*, solid) in Lac à la Croix on July 16–17, 1974.

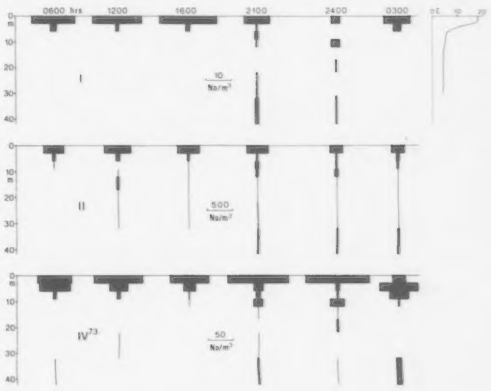

FIG. 6. Diurnal vertical migration of instars I, II, and IV[73] of *C. americanus* in Lac Gallienne on July 19–20, 1974.

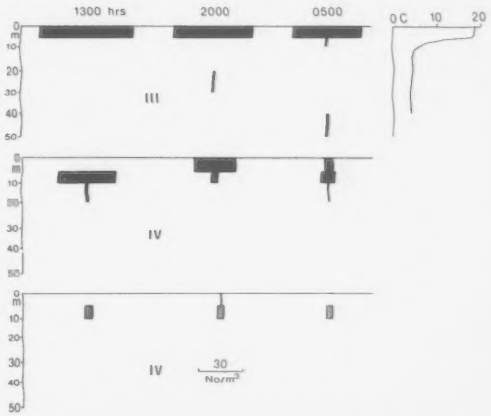

FIG. 7. Diurnal vertical migration of instars III and IV of *C. flavicans* (*above*, solid) and instar IV of *C. trivittatus* (*below*, hatched) in Lac Méchant on August 16–17, 1975.

Acknowledgments

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Rate of Conversion of ^{14}C-*p,p'*-DDT to *p,p'*-DDE by Brook Trout (*Salvelinus fontinalis*): Absence of Effect of Pretreatment of Fish with Compounds Related to *p,p'*-DDT[1]

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ADDISON, R. F., AND M. E. ZINCK. 1977. Rate of conversion of ^{14}C-*p,p'*-DDT to *p,p'*-DDE by brook trout (*Salvelinus fontinalis*): absence of effect of pretreatment of fish with compounds related to *p,p'*-DDT. J. Fish. Res. Board Can. 34: 119–122.

Brook trout (*Salvelinus fontinalis*) fingerlings were given four injections at weekly intervals of either *p,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD, or *p,p'*-DDMU to yield tissue concentrations of 1–5 mg/kg wet wt. The effect of these treatments on the subsequent dehydrochlorination of an injection of ^{14}C-*p,p'*-DDT was investigated. The rate of dehydrochlorination of ^{14}C-*p,p'*-DDT by the trout was not influenced by these treatments and was more highly correlated with factors such as the weight of the fish and their lipid content. Of the compounds injected during the treatment of the fish, *p,p'*-DDMU was cleared more rapidly than the others.

ADDISON, R. F., AND M. E. ZINCK. 1977. Rate of conversion of ^{14}C-*p,p'*-DDT to *p,p'*-DDE by brook trout (*Salvelinus fontinalis*): absence of effect of pretreatment of fish with compounds related to *p,p'*-DDT. J. Fish. Res. Board Can. 34: 119–122.

On a donné à des fingerlings d'omble de fontaine (*Salvelinus fontinalis*) quatre injections à intervalles d'une semaine soit de *p,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD ou *p,p'*-DDMU, donnant des concentrations tissulaires de 1–5 mg/kg de poids humide. Nous avons étudié l'effet de ces traitements sur la déshydrochloration subséquente d'une injection de ^{14}C-*p,p'*-DDT. Le taux de déshydrochloration de ^{14}C-*p,p'*-DDT par l'omble de fontaine n'est pas affecté par ces traitements, mais est plutôt en étroite corrélation avec des facteurs tels que le poids du poisson et son contenu en lipides. Parmi les composés injectés lors du traitement, *p,p'*-DDMU est éliminé plus rapidement que les autres.

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THE insecticide *p,p'*-DDT is dehydrochlorinated slowly by brook trout (*Salvelinus fontinalis*) to *p,p'*-DDE (Addison and Zinck 1975). Evidence from studies on other organisms suggests that the enzyme system(s) responsible may be induced by *p,p'*-DDT or other organochlorine compounds (Capdevilla et al. 1973; Terrière and Yu 1974). If this were also true for fish, the rate of their dehydrochlorination of *p,p'*-DDT could then depend on their residue burdens or on their history of exposure to other inducing compounds. This paper describes an experiment in which trout were injected with *p,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD, or *p,p'*-DDMU to yield tissue concentrations of a few milligrams per kilogram body weight, after which the rate of their dehydrochlorination of *p,p'*-DDT was measured.

[1]Bedford Institute of Oceanography Contribution.

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Materials and Methods

Labeled and unlabeled pesticides, chromatographic materials and solvents were obtained as described previously (Addison et al. 1972; Zinck and Addison 1975).

The experiment was divided into a treatment phase, during which the fish were injected with compounds related to *p,p'*-DDT, and an analytical phase, during which the rate of conversion of ^{14}C-*p,p'*-DDT to ^{14}C-*p,p'*-DDE was determined. In the treatment phase, brook trout (*S. fontinalis*) weighing from 12 to 61 g (but of the same age and from one hatchery stock) were randomly distributed among seven groups held in 90 × 90 cm fiberglass tanks (30 fish/tank) containing approximately 150 ℓ of dechlorinated fresh water flowing at approximately 2 ℓ/min. Water temperature was kept at 15°C and the fish were offered Ewoz fish chow ad lib. 3 times a week. Each fish in six of the groups was injected once a week, for 4 wk, with 5 μl ethanol or 5 μl ethanol containing 7.5 μg/μl of *p,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD, or *p,p'*-DDMU, according to the scheme in Table 1. All injections were made into the dorsal muscle, alternating from

TABLE 1. Treatment schedule and mortalities observed during experiment described in text. TP = treatment phase; AP = analytical phase.

Group	Injection in TP	Injection in AP	% mortality
1	Ethanol	Ethanol	nil
2	Ethanol	^{14}C-*p,p'*-DDT	13 (AP)
3	*p,p'*-DDT	^{14}C-*p,p'*-DDT	nil
4	*p,p'*-DDE	^{14}C-*p,p'*-DDT	nil
5	*p,p'*-DDD	^{14}C-*p,p'*-DDT	40(TP); see text
6	*p,p'*-DDMU	^{14}C-*p,p'*-DDT	10(TP)
7	nil	nil	nil

side to side. This regime was chosen so that the fish in groups 3–6 would accumulate compounds related to *p,p'*-DDT at concentrations of approximately 5 mg/kg wet wt (assuming a mean weight of 30 g at time of injection, and retention of the total injected dose). The seventh group was maintained under conditions similar to the others, but received no injections.

In the 5th wk (the start of the analytical phase) all fish in groups 2–6 received an injection of ^{14}C-*p,p'*-DDT (6.08 μCi/μM) in 5 μl ethanol, corresponding to body weight concentration of 0.25 ppm. Fish in group 1 received a further injection of 5 μl ethanol and those in group 7 received no injections. At intervals of 1, 14, 28, 42, and 56 days after this injection, five fish were taken from each group and were killed and analyzed for labeled and unlabeled organochlorine residues and metabolites generally as described previously (Addison et al. 1972; Addison and Zinck 1975; Zinck and Addison 1975).

Results

Deaths during the course of the experiment are summarized in Table 1. During the treatment phase, 12 fish (40%) jumped out of the tank housing the *p,p'*-DDD-treated group; since some of these showed the "pop-eye" symptom this response was attributed to supersaturation of gas. Reduction of the air supply to the tank prevented recurrence of the problem.

Mean fish weights showed no significant differences between groups sampled at the same time ($P > 0.05$ by *t*-test). There was a steady growth from a mean of approximately 30 g 1 day after injection with ^{14}C-*p,p'*-DDT to approximately 50 g 8 wk later. Lipid content remained steady at 5.6 ± 1.3% wet wt (mean ± SD of 156 determinations) and showed no differences between groups; there was, however, a general increase in lipid content with increasing body weight.

The fraction of total body weight accounted for by liver remained constant at 0.021 ± 0.004 (mean ± SD of 156 determinations) and showed no significant differences between treatments.

Control fish (groups 1 and 7) contained residues of *p,p'*-DDT, *p,p'*-DDD and *p,p'*-DDE at "background" concentrations of approximately 0.07, 0.03, and 0.07 ppm wet wt, respectively. Background concentrations in group 2 were similar after correction had been made to the GLC estimates of total residues present for the labeled component determined by scintillation counting.

FATE OF *p,p'*-DDT, *p,p'*-DDD, *p,p'*-DDE, AND *p,p'*-DDMU

Unlabeled pesticides injected during the treatment phase were retained in concentrations of between 1 and 5 mg/kg wet wt, in groups 3–6 depending on the weight of the fish and the time when it was analyzed. There was a steady decrease in the amount of injected residue retained throughout the analytical phase (Fig. 1A). For comparison between treatments, the data have been treated as conforming to linear regressions. We recognize that clearance of pesticides from fish may follow an exponential curve (e.g. Gakstatter and Weiss 1967; Grzenda et al. 1970) but since the fish were exposed to a repeated supply of pesticide it seemed simpler (and adequate) to illustrate the clearance by a linear regression. Unlabeled *p,p'*-DDMU was cleared from the fish appreciably faster than were the other three compounds. Comparison of the slopes of regressions by *t*-test showed that the differences were statistically significant ($P < 0.05$) in the case of *p,p'*-DDMU vs. *p,p'*-DDE and *p,p'*-DDT, but not in the case of *p,p'*-DDMU vs. *p,p'*-DDD because of the limited amount, and large variance, in the latter data.

Of the unlabeled pesticides injected during the treatment phase, only *p,p'*-DDT was metabolized to any extent; approximately 18% of what remained at the end of the analytical phase had been converted to *p,p'*-DDE.

^{14}C-*p,p'*-DDT METABOLISM

^{14}C-*p,p'*-DDT injected at the beginning of the analytical phase was cleared slowly during the following 8 wk. The rates of clearance (treated as

TABLE 2. Correlation matrix: regression of percent dehydrochlorination of ^{14}C-*p,p'*-DDT on various factors.

		x_1 Fish wt (*g*)	x_2 Lipid content (%)	x_3 Liver wt: body wt	x_4 Residue content (*mg/kg*)	*y* % Dehydrochlorination of ^{14}C-*p,p'*-DDT
y	% Dehydrochlorination of ^{14}C-*p,p'*-DDT	−0.730	−0.842	−0.341	−0.106	1.000
x_4	Residue content (*mg/kg*)	−0.349	−0.144	0.546	1.000	
x_3	Liver wt:body wt	0.139	0.200	1.000		
x_2	Lipid content (%)	0.773	1.000			
x_1	Fish wt (*g*)	1.000				

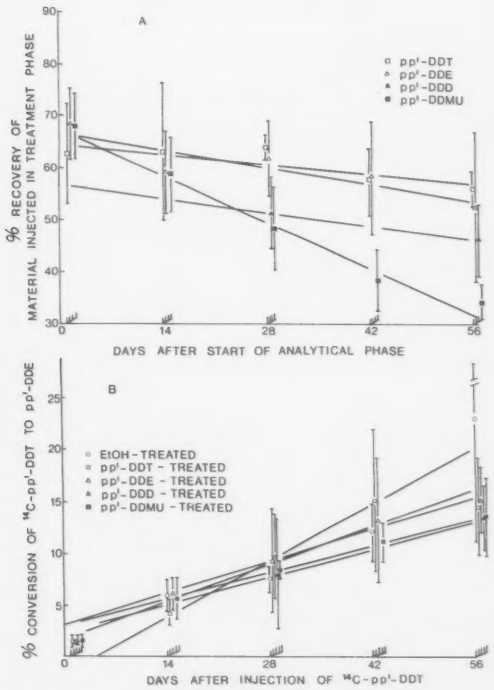

FIG. 1. A, Clearance during analytical phase of unlabeled *p,p'*-DDT-type compounds injected into brook trout (*Salvelinus fontinalis*) during treatment phase; B, percent dehydrochlorination of ^{14}C-*p,p'*-DDT by trout during analytical phase.

being linear to allow comparison with data for unlabeled *p,p'*-DDT clearance) did not differ significantly by *t*-test ($P > 0.05$) between treatment groups nor from the rate of clearance of unlabeled *p,p'*-DDT, injected during the treatment phase to group 3.

^{14}C-*p,p'*-DDT was converted to *p,p'*-DDE by fish from all groups; no other radioactive metabolites were detected. The conversion could be adequately described by a linear regression of percent conversion on time. The rate of conversion of ^{14}C-*p,p'*-DDT to *p,p'*-DDE was highest in control group 2 (Fig. 1B) but the only significant differences in rates ($P < 0.05$) were between the *p,p'*-DDMU-treated group (6) and the *p,p'*-DDT-treated and control groups (5 and 2).

Discussion

The experimental treatments did not appear to have any detrimental effects on the fish (Table 1). Apart from the large kill in the *p,p'*-DDD-treated group, apparently caused by supersaturation of gas, mortalities were infrequent and not consistently related to any experimental treatment. Furthermore, in all groups but group 2 (ethanol and ^{14}C-*p,p'*-DDT-treated) differences between mean weights at the start and end of the analytical phase were similar (70–100% increase). Group 2 contained two exceptionally small fish analyzed at the end of the experiment which reduced the mean weight of that group and increased its variance, but if they were ignored, mean weight differences in this group were similar to those in the others.

The rapid clearance of *p,p'*-DDMU injected during the treatment phase relative to the other members of the *p,p'*-DDT group of compounds was unexpected. Data obtained previously from a shorter experiment and showing more variance than those presented here did not demonstrate this difference (Addison and Zinck 1975). On the basis of purely physical considerations, there seems to be no reason why the behavior of *p,p'*-DDMU should differ from that of its congeners, since it also is a nonpolar, lipid-soluble organochlorine. Since it may occupy a central position in the eventual degradation of *p,p'*-DDT in animal systems (Peterson and Robison 1964) its anomalous behavior requires further study.

The conversion of ^{14}C-*p,p'*-DDT to *p,p'*-DDE was apparently not enhanced by pretreatment of the fish with other compounds in the DDT group.

The reduction in activity in the treated groups (3–6) below that in ethanol-treated controls (group 2) arose because of the unusually high rate of dehydrochlorination noted in the two very small fish in the latter group. This suggested that the rate of dehydrochlorination of ^{14}C-*p,p'*-DDT may have depended more on other factors than on pretreatment with compounds of the DDT group. A step-wise multiple linear regression supported this view; analysis of the data at the end of the analytical phase showed that the percentage of ^{14}C-*p,p'*-DDT dehydrochlorinated by each fish was correlated (inversely) with weight and lipid content (which were positively correlated) but to only a limited degree with either residue burden or liver weight to body weight ratios (Table 2). The final regression related percent dehydrochlorination (y) to body weight (x_1), lipid content (x_2), and residue burden (x_4) and had the form:

$$y = 38.59 - 0.11\,x_1 - 2.44\,x_2 - 2.14\,x_4 \quad (r = 0.91)$$

The absence of any effect of pretreatment with DDT-type compounds on the rate of ^{14}C-*p,p'*-DDT dehydrochlorination may have various explanations. First, the *p,p'*-DDT dehydrochlorinating systems in fish may simply not be inducible by such pretreatments, as they are in insects. Second, the compounds used in the treatment phase of the experiment may not have been inducers of the *p,p'*-DDT dehydrochlorinating system in fish. This would be unexpected, however, as those compounds that are effective inducers of mixed function oxidases in other systems are also inducers of insect *p,p'*-DDT dehydrochlorinating systems (Yu and Terrière 1972; Capdevilla et al. 1973), and *p,p'*-DDT and *p,p'*-DDE are in the former category (Bunyan et al. 1972). The possibility that ethanol (used as the vehicle for injection of the compounds in the treatment phase) masked any inductive effect of these compounds can be discounted for two reasons: there were no differences between liver weight to body weight ratios in ethanol injected (groups 1 and 2) and untreated controls (group 7) (suggesting no appreciable induction by ethanol itself); and the amounts of ethanol to which the fish were exposed (< 1 mg/g wt in toto over 5 wk) were considerably below those required to produce even a marginal induction of other mixed function oxidase enzymes in mammals (> 10 mg/g body wt daily for several weeks (Parke 1975). The most likely explanation of our data is therefore that the levels used in the treatment of our fish were below those required to stimulate a response in the *p,p'*-DDT dehydrochlorinating system.

The doses of organochlorine compounds with which our experimental fish were treated during the treatment phase of the experiment are similar to those found in environmental samples from various locations in North America. Although intramuscular injection of these compounds may not mimic exactly the distribution attained by natural uptake routes (cf. Addison et al. 1976), our data suggest that such residue burdens do not interact with each other's metabolism.

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Genetic Differences in Growth and Survival of Juvenile Hatchery and Wild Steelhead Trout, *Salmo gairdneri*

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REISENBICHLER, R. R., AND J. D. MCINTYRE. 1977. Genetic differences in growth and survival of juvenile hatchery and wild steelhead trout, *Salmo gairdneri*. J. Fish. Res. Board Can. 34: 123–128.

Relative growth and survival of offspring from matings of hatchery and wild Deschutes River (Oregon) summer steelhead trout, *Salmo gairdneri*, were measured to determine if hatchery fish differ genetically from wild fish in traits that can affect the stock–recruitment relationship of wild populations. Sections of four natural streams and a hatchery pond were each stocked with genetically marked (lactate dehydrogenase genotypes) eyed eggs or unfed swim-up fry from each of three matings: hatchery × hatchery (HH), hatchery × wild (HW), and wild × wild (WW). In streams, WW fish had the highest survival and HW fish the highest growth rates when significant differences were found; in the hatchery pond, HH fish had the highest survival and growth rates. The hatchery fish were genetically different from wild fish and when they interbreed with wild fish may reduce the number of smolts produced. Hatchery procedures can be modified to reduce the genetic differences between hatchery and wild fish.

REISENBICHLER, R. R., AND J. D. MCINTYRE. 1977. Genetic differences in growth and survival of juvenile hatchery and wild steelhead trout, *Salmo gairdneri*. J. Fish. Res. Board Can. 34: 123–128.

Nous avons mesuré la croissance et la survie relatives de descendants issus de croisements entre des truites steelhead de pisciculture et des steelheads d'été sauvages, *Salmo gairdneri*, de la rivière Deschutes (Orégon), afin de déterminer si les poissons de pisciculture diffèrent génétiquement des poissons sauvages par des caractères susceptibles d'affecter la relation stock–recrutement des populations sauvages. Des sections de quatre cours d'eau naturels et un étang de pisciculture ont été ensemencés chacun avec des œufs embryonnés génétiquement marqués (génotypes lactate déshydrogénase) ou avec des alevins non nourris au stade de nage vers le haut résultant de chacun de trois croisements : pisciculture × pisciculture (HH), pisciculture × sauvage (HW) et sauvage × sauvage (WW). Dans les cours d'eau, les poissons WW ont le plus haut taux de survie, et les poissons HW le plus haut taux de croissance là où il y a des différences significatives; dans l'étang de pisciculture, les poissons HH ont les taux de survie et de croissance les plus élevés. Les poissons de pisciculture diffèrent génétiquement des poissons sauvages et, quand ils se croisent avec des poissons sauvages, peuvent causer une diminution du nombre de smolts produits. Les méthodes de pisciculture peuvent être modifiées de façon à réduire les différences génétiques entre les poissons de pisciculture et les poissons sauvages.

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INTRODUCTION of artificially propagated salmonids into natural stream systems may influence resident wild populations (Hochachka 1961). The hatchery-reared fish may affect the wild population through competition for food and space resources (Needham and Slater 1944; Reimers 1957; Vincent 1972), or the genetic structure of the wild population may be affected by interbreeding of hatchery fish and wild fish on natural spawning grounds. A genetic effect is contingent upon there being a genetic difference between the wild fish and the hatchery fish spawning together in the wild. Results of studies with brook trout, *Salvelinus fontinalis* (Greene 1952; Flick and Webster 1964), and with Atlantic salmon, *Salmo salar* (Fenderson et al. 1968), indicated the existence of genetic differences between hatchery and wild fish.

For anadromous species on the Pacific coast, it

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is not known whether adults resulting from release of artificially propagated juveniles differ genetically from wild adults, especially in traits that may affect the stock–recruitment relationship of a wild population (Ricker 1975). This study was designed to test the hypothesis that there are no genetic differences in growth rate or survival between offspring from matings of hatchery × hatchery (HH), hatchery × wild (HW), and wild × wild (WW) summer steelhead trout, *S. gairdneri*.

Materials and Methods

Summer steelhead trout were captured during their upstream migration in a trap at Pelton reregulating dam on the Deschutes River, a tributary of the Columbia River, during fall and winter 1974–75. They were held at the Oregon Department of Fish and Wildlife Round Butte Hatchery until sexually mature. Hatchery fish could be identified because they are finclipped before release into the Deschutes River. As the terms are used in this paper, hatchery fish are reared in a hatchery for about 1 yr before their release, and wild fish result from natural reproduction.

Eyed eggs or unfed swim-up fry from matings of these adults subsequently were placed in four small streams in the upper Trout Creek drainage which flows into the Deschutes River. Average weekly flows in these streams exceeded 28 ℓ/s only during periods of snowmelt or unusually wet weather. Water temperatures ranged from 0°C during winter and spring to 26°C during summer. Steelhead and resident rainbow trout and longnose dace, *Rhinichthys cataractae*, are known to occur in these streams. In January 1975 screens were installed in these streams to delineate four study sections (one each, 1.6 km long, in Opal, Dutchman, and Potlid creeks; and one, 0.8 km long, in Trout Creek) and to prevent steelhead and resident rainbow trout from entering the sections for spawning. Trout longer than 6.5 cm were removed from the sections by repeated electrofishing. Downstream traps were installed at each screen to capture out-migrating fish.

On February 24, 1975, each adult was killed, its eggs or sperm placed in a numbered container and stored at 4°C and a sample of liver tissue analyzed electrophoretically to determine the lactate dehydrogenase-4 (LDH-4) genotype of each fish (Utter et al. 1973). Preliminary analyses indicated the occurrence of three LDH genotypes, *BB*, *B′B*, and *B′B′*, which are known to exhibit simple Mendelian inheritance (Morrison and Wright 1966). After the genotypes were determined, the stored gametes were combined into three sets of fertilized eggs. Each set consisted of groups of eggs of each LDH genotype and each mating as follows:

	Matings		
Sets	HH	HW	WW
I	*B′B′*	*B B*	*B′B*
II	*B B*	*B′B*	*B′B′*
III	*B′B*	*B′B′*	*B B*

The numbers of adults used in these matings were:

Hatchery				Wild			
Males		Females		Males		Females	
B B	*B′B′*	*B B*	*B′B′*	*B B*	*B′B′*	*B B*	*B′B′*
13	14	13	16	4	4	4	4

Non-LDH genotypic variability was maximized in the offspring by dividing the eggs from each female into a number of subsamples equal to the number of males to be used in the mating. Each subsample of eggs was then fertilized by sperm from one male. Thus, a mating representing m males and f females consisted of $m \times f$ individual matings.

It was necessary to repeat the spawning procedure on a second date (March 18) because of low survival in one mating of set III. Matings for sets II and III were repeated at this time. The numbers of adults used in these matings were:

Hatchery				Wild			
Males		Females		Males		Females	
B B	*B′B′*	*B B*	*B′B′*	*B B*	*B′B′*	*B B*	*B′B′*
13	14	13	20	5	12	4	6

Fertilization was delayed for < 12 h for all matings on both dates. The fertilized eggs were placed in Heath® incubator trays with a 23–27 ℓ/min flow of 10°C water. The eggs were left undisturbed until they reached the eyed stage; dead eggs were then removed.

Eggs from both spawning dates were used to stock the study sections (Table 1). Vibert® boxes, each containing 600 eggs, were marked to identify the eggs they contained, and three boxes of eggs (from each mating: HH, HW, WW) were placed in artificial redds excavated at regular intervals in the substrate of the study sections in Dutchman (15 redds), Potlid (15), and Trout (10) creeks. Each depression was then filled with gravel 1.5–7 cm in diameter. Potlid and Dutchman creeks were stocked with 9,000 eggs from each mating and Trout Creek with 6,000 from each. The Vibert® boxes were removed from the redds after fry emergence from the gravel was complete, and dead embryos were counted.

Opal Creek was stocked with 22,500 swim-up fry and a hatchery pond at Round Butte Hatchery with 18,000 (Table 1). The fry stocked in the hatchery pond were reared according to standard practices by personnel at Round Butte Hatchery.

Samples of juvenile fish were captured from the sections by electrofishing several times during the period from July 15, 1975 to April 8, 1976. These samples and the fish removed from the traps were frozen and taken to the laboratory where they were thawed and measured (fork length). The LDH genotype of each fish was determined from eye-tissue homogenates.

Results

Among eyed eggs from the three streams, survival of WW embryos was significantly greater ($P < 0.05$) than that of HH and HW embryos

TABLE 1. Lactate dehydrogenase genotypes of eyed eggs (E) of steelhead trout (*Salmo gairdneri*) placed in artificial redds and unfed swim-up fry (F) stocked in experimental streams and a hatchery pond. HH = hatchery × hatchery; HW = hatchery × wild; WW = wild × wild.

Steelhead stocked		Matings		
Location	Life stage	HH	HW	WW
Potlid Creek[a]	E	*B′B′*	*B B*	*B′B*
Hatchery Pond[a]	F	*B B*	*B′B*	*B′B′*
Dutchman Creek[b]	E	*B B*	*B′B*	*B′B′*
Trout Creek[b,c]	E	*B′B*	*B′B′*	*B B*
Opal Creek[b,c]	F	*B′B*	*B′B′*	*B B*

[a]Eggs fertilized February 24, 1975.
[b]Eggs fertilized March 18, 1975.
[c]Opal and Trout creeks stocked with fish from the same matings.

(Table 2). (These data did not include one Vibert® box of WW embryos from Potlid Creek and five boxes — two HH, two HW, and one WW — from Trout Creek that had been washed out of their redds.) The variance of the arc sine square root of percentage mortality for groups of eyed eggs was analyzed following the technique of least squares analysis of a linear model (Draper and Smith 1966) to overcome the problem of unequal sample sizes.

The traps at the lower ends of each study section were functional and assumed to have captured all downstream migrants during the period from their installation in April to November 8, 1975, except in Trout Creek where high water caused the trap to be inoperative during portions of May and June. The traps were not functional for most of the period after November 8, 1975. Less than 500 experimental fish from each stream were captured in the traps (Table 3).

In all streams where the numbers of juveniles from the experimental matings differed significantly, progeny of WW matings were most abundant (Table 4). In the hatchery pond, HH fish were the most abundant. Overwinter (October–March) shifts in relative abundance occurred in Opal Creek, where survival of WW fish was significantly greater than that of HH fish in the fall but not in the spring. In Potlid Creek, frequencies in the fall sample indicated no significant difference in relative abundance, and frequencies in the spring sample indicated significantly greater survival of WW than of HH fish.

TABLE 2. Percent survival for different groups of eyed eggs stocked in three experimental streams (HH = hatchery × hatchery; HW = hatchery × wild; WW = wild × wild). *9,000/mating; **6,000/mating.

Creek	Total no. stocked	Matings		
		HH	HW	WW
Potlid	27,000*	89	83	93
Dutchman	27,000*	70	72	78
Trout	18,000**	75	84	86
Combined creeks	72,000	78.4	79.5	86.1

TABLE 3. Frequency of fish from three experimental matings that were recovered from traps before October 1975.

Creek	Matings			
	HH	HW	WW	χ^{2a}
Opal	135	121	120	1.1
Potlid	52	228	105	141.1[b]
Dutchman	122	99	190	23.4[b]
Trout	75	88	81	2.4

[a]Expected values for the chi-square calculations were derived by using data from Table 3 and assuming that eggs from dislocated Vibert® boxes made no contribution to the experimental populations.
[b]Statistically significant at the 0.005 level.

Out-migration rates of 0% for HH, 31% for HW and 69% WW would have produced the observed frequency change between fall and spring in Opal Creek. The only trap sample obtained after September, which was from Opal Creek on December 1, 1975, consisted of only eight fish — two HW and six WW. Deschutes River summer steelhead trout fry from tributary streams (Fessler 1974) and summer steelhead and resident rainbow trout from several Idaho streams (Bjornn 1971) are known to migrate downstream during the fall and winter. A differential out-migration in Opal Creek but not in Trout Creek, both of which contained fish from the same matings, may have been due to the more extreme winter conditions in Opal Creek. Trout and Potlid creeks were covered with ice during most of the winter, but Opal Creek often was not. The recurrent absence of a protective covering of ice resulted in greater fluctuations in temperature in Opal Creek and ice frequently formed on the stream bottom.

In Opal and Potlid creeks, HW fish were larger ($P < 0.005$) than HH and WW fish (Table 4). Mean lengths of fish from Dutchman and from Trout creeks were not significantly different. In the hatchery pond, the HH fish were significantly larger than the HW and WW fish ($P < 0.01$).

Differences in the environmental conditions from stream to stream and sampling error resulting from the relatively small numbers of wild adults used in the matings were possible causes for the lack of consistency observed in the relative

TABLE 4. Frequencies (percent) and mean lengths (millimeters) in parentheses, of fish from experimental matings in samples from streams and a hatchery pond. χ^2 compares numbers of fish from each mating.

Location and collection date	No. fish	Matings			χ^{2a}
		HH	HW	WW	
Opal Creek					
July 14, 1975	222	34(46)	29(46)	37(45)	2.0
September 9, 1975	159[b]	26(59)	32(62)	42(59)	5.6[c]
October 25, 1975	464	26(60)	30(63)	44(61)	27.5[c]
March 3, 1976	528	33(63)	32(65)	35(63)	0.8
Potlid Creek					
September 9, 1975	130	31(54)	37(57)	32(55)	0.7
November 4, 1975	316	39(59)	31(60)	30(60)	2.6
March 3, 1976	296	28(62)	33(65)	39(63)	7.2[c]
Dutchman Creek					
August 17, 1975	453	33(55)	33(54)	34(54)	0.1
November 7, 1975	422	32(62)	36(63)	32(61)	2.7
February 29, 1976	234	29(70)	37(68)	34(70)	2.6
Trout Creek					
November 4, 1975	435	23(51)	29(52)	48(51)	27.4[c]
April 8, 1976	115	22(54)	36(52)	42(52)	7.8[c]
Hatchery Pond					
August 16, 1975	497	40(60)	31(56)	29(56)	10.8[c]

[a]Expected values were calculated by assuming that embryos from dislocated boxes made no contribution to the experimental populations.
[b]Measured lengths of 158 fish.
[c]Statistically significant at the 0.05 level.

lengths and abundances of fish in the different study sections. Analysis of the variance in length for fish obtained in the fall and spring from all streams and inspection of the relative frequency data suggested that there was no selective advantage associated with individual LDH genotypes of fish that were stocked in the streams.

Discussion

There were genetic differences in growth rate and survival between the offspring of hatchery and wild steelhead. Differences in the numbers of fish recovered from the traps, although not consistent, suggested that there were behavioral differences between offspring of hatchery and wild fish; however, the effect of migration differences on the relative growth and survival of fish from these matings was not clear.

The observed differences in survival suggested that the short-term effect of hatchery adults spawning in the wild is the production of fewer smolts and ultimately, fewer returning adults than are produced from the same number of only wild spawners. This effect depends on the particular limiting factors and environmental conditions in a given stream system and on the total number of spawners. In some stream systems, the same total number of smolts may be obtained simply by allowing more hatchery adults to spawn (Fig. 1A), whereas in other systems, numbers of smolts may be reduced regardless of how many hatchery adults spawn (Fig. 1B).

The length data for fish from Potlid and Opal creeks indicated that there were genetic differences between offspring from the different matings surviving after 1 full yr of exposure to natural selection in the stream environment. If these and other genetic differences persist until the offspring of hatchery and wild fish return as adults, there will be an additional effect on the wild population. The expected result is a reduction in the overall reproductive success of the wild population (long-term effect in Fig. 2). The relative magnitude of short-term and long-term effects would be expected to vary through time. In years in which the environment is unusually favorable for survival of fish, the short-term effect would be expected to be reduced, but this reduction in turn would increase the long-term effect in later years when the "mild year offspring" returned as adults and spawned in the stream. Conversely, in years in which the environment is unusually harsh, the short-term effect would be expected to be high, and the long-term effect low.

The results may provide only a conservative

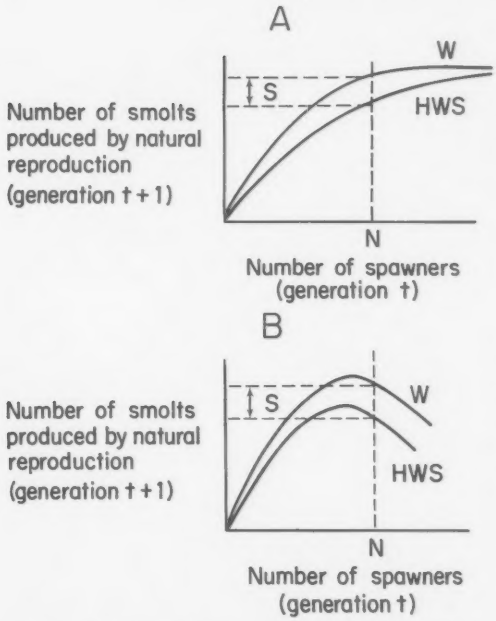

FIG. 1. Hypothetical stock–recruitment relationships. Numbers of smolts produced at high spawner densities are equivalent (A); never equivalent (B). See text for explanation. Short-term effect at an escapement of N spawners is S. W = the relationship for all wild spawners with no previous hatchery introductions. HWS = the relationship due to hatchery adults interbreeding with wild adults. HWS varies with the proportion of the total spawners that are hatchery fish; this proportion increases the distance between HWS and W. Available spawning area not limited (A); limited (B).

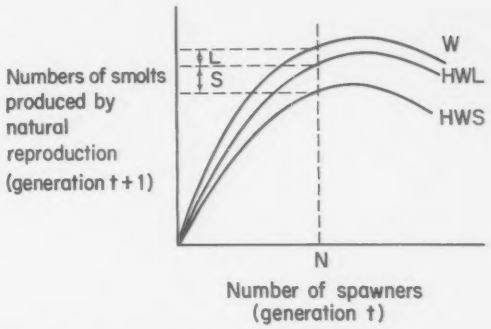

FIG. 2. Suggested effects of interbreeding between hatchery and wild fish and a hypothetical stock–recruitment relationship for naturally reproducing fish. Total effect at an escapement of N spawners is L + S. W = the relationship for all wild spawners with no previous hatchery introductions; HWL = the relationship resulting from genetic differences between wild adults that are offspring of hatchery and wild fish; HWS = the relationship resulting from interbreeding of hatchery wild adults. S = short-term effect and L = long-term effect (see text for explanation).

estimate of the effects of interbreeding between hatchery and wild fish. Most of the adult hatchery fish captured in the 1974–75 winter were no more than two generations removed from wild steelhead trout parents. As a stock of fish is subject to the selective forces of the hatchery environment over more generations, the genetic difference between the hatchery fish and the original wild stock will probably increase. In addition, a large number of adult hatchery fish spawn in the river immediately below the Pelton reregulating dam; consequently, some of the wild fish used for this experiment are likely to have had a hatchery fish for a parent. Finally, making all matings for each stream in the hatchery on the same day eliminated any potential effects due to differences in adult behavior. Differences in spawning time and other adult behavior may have a considerable effect on the relative performance of offspring of hatchery and wild adults. Hatchery steelhead from the Deschutes River tend to spawn earlier than the wild fish. If natural selection in the stream system is regulating spawning time for optimal survival, then in most years poorer survival would be expected from fish spawning earlier than wild fish.

The smaller the genetic difference between the hatchery fish and wild fish the smaller the expected effect on the wild population. The genetic difference can be reduced by using native wild fish which are adapted to that particular stream system for brood stock to initiate the hatchery program and in subsequent generations. Hatchery practices could be modified to reduce artificial selective pressures such as selection for early time-of-return and rapid growth in the hatchery. These modifications extend the period of egg collection and prevent destruction of slow-growing individuals (grade-outs). This decrease in efficiency represents the cost of reducing the genetic differences between hatchery and wild fish.

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NOTES

Inconnu, *Stenodus leucichthys*, Migration Studies in Alaska 1961–74

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ALT, K. T. 1977. Inconnu, *Stenodus leucichthys*, migration studies in Alaska 1961–74. J. Fish. Res. Board Can. 34: 129–133.

During studies on inconnu, *Stenodus leucichthys*, in the Kobuk, Yukon, Koyukuk, Kuskokwim, Holitna, and Chatanika rivers, and the Selawik and Minto Flats areas, 3907 fish were marked. Nine hundred and ten (23.3%) had been recovered by December 31, 1974.

Tag return data indicate that tagged inconnu comprise four major populations: Kobuk–Selawik, lower Yukon, Kuskokwim, and Minto Flats.

Inconnu undertake rapid downstream postspawning migrations to wintering areas in lower reaches of large rivers (Yukon, Kuskokwim) or slightly brackish inlets (Hotham Inlet, Selawik Lake), then begin slow upstream migration before spring ice breakup, with nonspawning fish travelling to summer feeding areas and spawners migrating to spawning grounds during summer and fall. Minto Flats fish are nonanadromous.

Three fish, tagged in the lower Yukon River and recaptured in the Koyukuk River, migrated upstream 1035–1156 km, averaging 13.8 km/day. The longest migration recorded was nearly 1,600 km.

ALT, K. T. 1977. Inconnu, *Stenodus leucichthys*, migration studies in Alaska 1961–74. J. Fish. Res. Board Can. 34: 129–133.

Au cours d'études sur l'inconnu, *Stenodus leucichthys*, dans le fleuve Yukon et les rivières Kobuk, Koyukuk, Kuskokwim, Holitna et Chatanika, et dans la région des Terres-basses de Selawik et de Minto, 3907 poissons ont été marqués. Au 31 décembre 1974, 910 (23.3%) avaient été recapturés.

Les retours d'étiquettes indiquent que les inconnus marqués constituent quatre populations majeures: Kobuk-Selawik; bas-Yukon; Kuskokwim; et Terres-basses de Minto.

L'inconnu entreprend de rapides migrations en aval après la fraie vers des régions d'hivernement dans le cours inférieur des grands cours d'eau (Yukon, Kuskokwim) ou dans des baies légèrement saumâtres (inlet Hotham, lac Selawik). Il commence ensuite une lente migration en amont avant la débâcle de printemps, en compagnie de non-reproducteurs se rendant sur leurs lieux d'alimentation estivale et de reproducteurs remontant vers les frayères en été en en automne. Les poissons des Terres-basses de Minto sont non anadromes.

Trois poissons marqués dans le bas-Yukon et recapturés dans la rivière Koyukuk avaient parcouru en amont 1035–1 156 km, à une vitesse moyenne de 13.8 km/jour. Le plus long trajet enregistré a été de près de 1 600 km.

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THIS study, accomplished by a tag and recovery program, was undertaken to determine migration routes and timing of inconnu, *Stenodus leucichthys*, in Alaska. Other aspects of the life history of the inconnu, including spawning, general observations on migrations, age and growth, and utilization have been published (Alt 1969, 1973).

In Alaska, inconnu are found in the drainages of the Yukon, Kuskokwim, Kobuk, and Selawik rivers where they support important subsistence fisheries and small but potentially valuable sport and commercial fisheries. Since inconnu travel great distances during their migrations, it is necessary to clearly define their routes so that equitable allocations of each population can be made among the three user groups.

Very little is known of inconnu movements in North America other than observations that large-scale movements of inconnu occur during certain times of the year. In a Canadian tagging study on Great Slave Lake (Keleher 1963), 23 inconnu were marked and 4 recovered, all in approximately the same area. Russian researchers appear to

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have complete documentation of migrations and population separation of inconnu in the Siberian rivers (e.g. Vork 1948a, b, Ob and Yenesei rivers; Menshikov 1935, Irtysh River; Podlesnyi 1941, Caspian Basin; Novikov 1966, Kolyma River).

Materials and methods — The study was conducted from 1961 to 1974, and 3907 inconnu were tagged throughout their major Alaskan range except for the upper Yukon River (Fig. 1). More fish were tagged in the Kobuk River and Selawik area (63%) than in the rest of the state.

All 1326 Kobuk River inconnu were tagged on spawning grounds above Kobuk village in September of 1967–71 (Fig. 2). Selawik tagging operations, conducted in June on feeding fish, included tagging 1013 fish at Tuklomarak River mouth, 200 in Inland Lake, 55 on the northeast shore of Selawik Lake, and 21 at Selawik River mouth (Fig. 2, inset).

Lower Yukon River inconnu were tagged in various locations in the lower river during June of 1961–66 and 1974. Koyukuk River fish were tagged along spawning migration routes and on spawning grounds in September in the main Koyukuk River and in the Alatna River.

Inconnu in the lower Kuskokwim River were tagged in June of 1962–66 near Bethel, 140 km upstream from the Kuskokwim mouth. Other fish tagged in the middle and upper Kuskokwim River included 452 tagged in the lower 32 km of the Holitna River, 16 at the mouth of the Tatlawiksuk River, and 52 on the spawning grounds located in the lower 200 m of Highpower Creek, 1280 km upstream from the mouth of the Kuskokwim River.

Minto Flats tagging operations were conducted during June and July of 1967–69 in the extensive lake and slough area near the Tolovana–Chatanika, and Chatanika–Tatlina river confluences and during September near the spawning grounds in the upper Chatanika River.

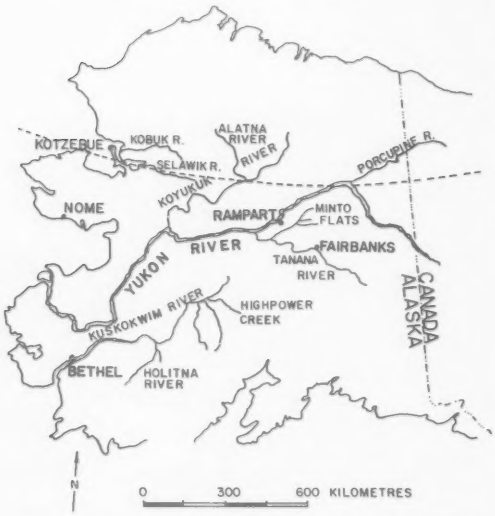

FIG. 1. Location of major drainages in Alaska containing inconnu (*Stenodus leucichthys*). Map reproduced from U.S.G.S. Alaska Map C.

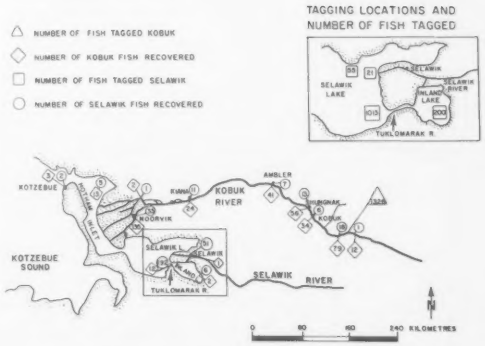

FIG. 2. Inconnu tag and recovery locations, Kobuk–Selawik areas, Alaska.

Fish were captured by set and drift gillnets, beach seine, fishwheel, weir, and hook and line. Fish tagged ranged in fork length from 238 to 1269 mm. The majority were tagged with spaghetti tags, but Petersen disc tags were used on the Holitna River fish in 1967 and 1968, and Floy internal anchor tags were used on the Kobuk River in 1971. Tags were inserted through the musculature immediately posterior to the dorsal fin.

Since most tagging was done in remote areas, success of the project depended upon Indian and Eskimo subsistence fishermen returning tags. Excellent cooperation was shown due to payment of $1.00 reward for each tag returned, and an information dissemination program. Approximately 15% of the tag recoveries were made by Alaska Department of Fish and Game tagging crews in subsequent years, and < 5% were returned by commercial fishermen in the Kotzebue area and sport fishermen in the Minto Flats and Holitna River areas. Frequent visits to some villages revealed that a few recovered tags had been lost. There was also an unknown mortality from tagging that could result in a higher actual recovery percentage than that recorded. Tagging data analysis was aided by an IBM 360 computer.

Results and discussion — A total of 910 or 23.3% of the tagged inconnu were recovered by December 31, 1974, and comprised four major populations: Kobuk–Selawik, lower Yukon River, Kuskokwim River, and Minto Flats.

Kobuk River–Selawik — Inconnu found in the Kobuk River, Kotzebue Sound, and the Selawik River–Selawik Lake area constitute a single population with spawning grounds located in the upper Kobuk and upper Selawik rivers. Wintering

areas and major feeding areas are in Hotham Inlet, Selawik Lake, Inland Lake, and the lower reaches of the Selawik and Kobuk rivers.

Of the Kobuk River tagged fish, 414 (31%) have been recovered, including 281 captured during the postspawning downstream migration during October and November of the same year as tagged. Tag returns from the five villages along the Kobuk River indicated a fairly fast migration down to Kiana and Noorvik and then a slower migration to the wintering areas. Many of the recoveries made at Noorvik were from under-ice gillnet sets in early November. Of 134 Kobuk River tagged fish that reached the wintering grounds, 103 were recovered on the Kobuk River in subsequent years, 16 were recovered in the Hotham Inlet–Kotzebue area, and 15 were recovered in the Selawik area. Duration of the upstream spawning migration is longer than the postspawning migration but generally the run is near Kiana by late June and reaches the spawning grounds above the village of Kobuk from late July through late September. Inconnu do not enter the tributaries of the Kobuk River.

The migration timing of Selawik fish is similar to that of Kobuk River fish. This includes a movement of prespawning fish up the Kobuk or Selawik river for about a month after breakup; early movements of feeding fish into the extensive lake and slough areas of the lower Kobuk River, Selawik River, Tuklomarak River, and Inland Lake; August and September movement of feeding fish into Selawik Lake; and a late October through December under-ice movement of spent fish down the lower Selawik River to Selawik Lake.

Recoveries of 346 Selawik tagged fish were made, including 249 (72%) in the Selawik area, 90 (26%) in the Kobuk River, and 7 (2%) in the Kotzebue area. This indicates that the Selawik area is an important feeding and overwintering location for fish that spawn in the upper Kobuk River.

Because of logistical problems no inconnu were collected from spawning grounds located in the upper Selawik River, so it is not known if inconnu that spawn in the Selawik River one year might spawn in the Kobuk River in subsequent years, or if the subpopulations are somewhat discrete. An aerial survey of the upper Selawik River spawning grounds in September 1968 gave a minimum count of 1428 fish, while a survey in late August 1971 gave a minimum count of 1020 fish. These counts are approximately 20% of the mean 1967–71 aerial counts of Kobuk River spawning ground inconnu. The difference in numbers of spawning fish is probably due to the Kobuk River's larger size and greater amount of spawning habitat.

Evidence for a single population theory is reinforced by similar growth patterns of Selawik and Kobuk fish (Alt 1973).

Lower Yukon River–Koyukuk River — Inconnu found in the Koyukuk River, the lower Yukon River, and the Yukon River near Rampart constitute a single population termed lower Yukon River inconnu. A slower growing group of inconnu inhabits the upper Yukon River near the Canadian border and probably constitutes another population. Fifty-five of the 439 inconnu tagged in the lower Yukon River were recovered (Fig. 3). Recovery locations of these fish were mainly in the lower Yukon River and along spawning migration routes in the Koyukuk River and the Yukon River near Rampart. Recoveries were made in the same year as fish were tagged and in subsequent years.

Twelve of the 96 inconnu tagged in the vicinity of the Koyukuk River spawning grounds were recaptured. These recoveries were made along migration routes in the Koyukuk River and in the lower Yukon River.

Migration timing of lower Yukon River inconnu is as follows: the fish arrive in the vicinity of the Koyukuk River spawning grounds near Hughes in late August and September and at the Alatna River spawning grounds by mid- to late September. Those spawning in the Yukon River above Rampart village migrate past Rampart during late August to early October, with the peak of the run occurring in early September. The Koyukuk, Alatna, and Yukon rivers are often freezing during the spawning period and postspawning downstream migration; consequently, few inconnu are taken by subsistence fishermen during October,

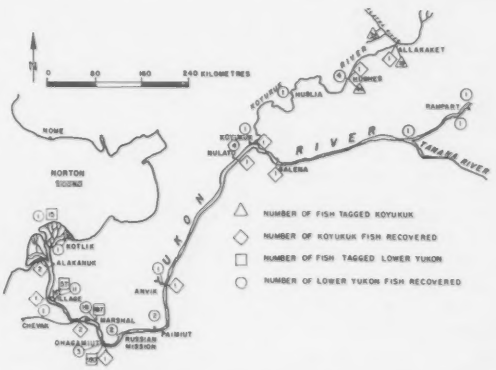

FIG. 3. Inconnu tag and recovery locations, Yukon River system, Alaska.

and no tagged fish were captured. In November, spent fish are taken in various areas of the Yukon River from Nulato to the mouth of the Yukon. All December recoveries were made in the lower 320 km of the Yukon River except for one fish recovered December 15 at Nulato that had been tagged 80 days previously 88 km up the Alatna River. It is probable that the entire population does not overwinter in brackish water at the mouth of the Yukon but rather is distributed along the lower 200–300 km of the river, with the largest concentrations reaching the mouth of the Yukon in January, February, and March.

Upstream movement begins under the ice in April and May. In June and July the fish are widely distributed and many spawners have already entered the Koyukuk River, while most nonspawners and immature fish remain in the lower section of the Yukon River to feed.

Inconnu of the lower Yukon population have the longest recorded migrations in Alaska. The distance from the Yukon River mouth to spawning grounds located 88 km up the Alatna River is 1602 km (1001 mi). One tagged fish had traveled nearly this distance, while other tagged fish had travelled between 1280 and 1360 km. Inconnu in the great Siberian rivers, the Ob and Lena, travel up to 2000 km on their spawning migrations (Vork 1948a, b; Kirilov 1962).

Upstream migration rate in the Yukon River was determined from three fish tagged at Ohagamiut in June and recovered in August and September. These fish traveled 1035 to 1156 km from the tagging site to capture locations on the Koyukuk River spawing grounds and averaged 13.8 km per day.

Lower Kuskokwim–Holitna rivers — Tag return data from the lower Kuskokwim River and the Holitna River indicate that these fish constitute a single population, termed Kuskokwim River inconnu. Fifty-seven of the 500 Kuskokwim tagged fish were recovered, mainly along migration routes and feeding areas on the Kuskokwim River (Fig. 4).

The general migration pattern of Kuskokwim River inconnu is a slow downstream movement past Kalskag during early winter, with major concentrations located near villages below Bethel and possibly Kuskokwin Bay after January 1. Before ice breakup, the fish begin moving upstream. At breakup in mid- to late May, tags have been recovered by residents of Kalskag, Aniak, Crooked Creek, and Sleetmute. In early June 1971, inconnu began appearing in gillnet catches 32 km up the Holitna River. Some of these fish remain all summer and feed in the Holitna River, while others feed only during

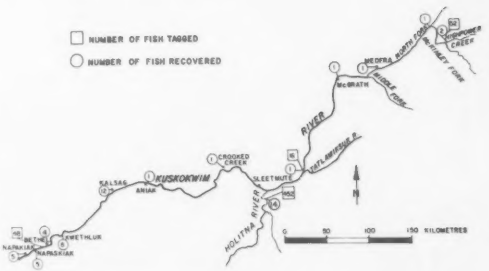

FIG. 4. Inconnu tag and recovery locations, Kuskokwim River, Alaska.

early summer, then leave the Holitna River and migrate farther up the Kuskokwim River in July and August. Inconnu, nearly 100% spawners, reach McGrath and Medfra from July through August and are taken in subsistence gillnets. The fish reach the spawning grounds in the lower reaches of Highpower Creek in September. In 1971 spawning occurred from September 29 through October 4. The major portion of the downstream postspawning migration is not documented because of freeze-up.

One fish, tagged 32 km up the Holitna River in 1968, was recovered below Nulato in the Yukon River in 1973. This recovery is not included in Fig. 4. This is the first record of an inconnu migrating from one major drainage in Alaska to another. The distance is approximately 1700 km if the fish traveled to the mouth of the Kuskokwim, then up to the Yukon River. It is also possible to reach the Yukon River via the extensive lake and slough system between the two rivers in years of high water.

No tagged inconnu were recovered in the estuarine Kuskokwim Bay, probably due to lack of fishing effort. Rae Baxter (Alaska Department of Fish and Game, Bethel) reported taking inconnu during winter test netting operation in Kuskokwim Bay.

Minto Flats–Chatanika River — Inconnu tagged in the Minto Flats area and in the upper Chatanika River belong to the same population, termed Minto Flats inconnu.

Of the 168 inconnu tagged in the Minto Flats and Chatanika River 27 were recaptured, including 6 of the Minto Flats tagged fish that were recovered on the spawning ground, and 2 tagged on the Chatanika spawning ground that were recovered in the Minto Flats (Fig. 5).

The fall and winter migration patterns of Minto Flats inconnu are not fully understood but it is believed that they overwinter in the lower Tolovana and Tanana rivers, as the Minto Flats waters

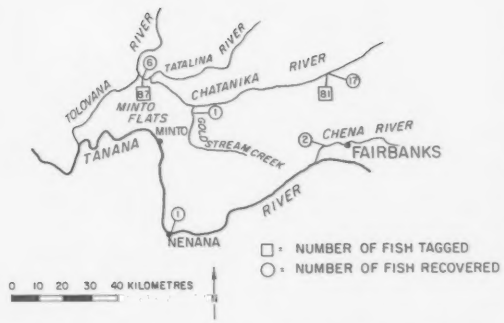

FIG. 5. Inconnu tag and recovery locations, Minto Flats, Alaska.

become oxygen deficient, and no fish have been captured in these waters after December. Inconnu enter the Minto Flats in late May after breakup. The Minto Flats area is the main summer feeding and rearing area. The spawning population begins migrating up the Chatanika River in late June and reaches the area of the spawning grounds located approximately 130 km up the Chatanika River in late August and September. Spawning occurs in late September and early October, after which the fish migrate downstream.

Recovery of one tagged fish in the Tanana River at Nenana and two in the Chena River indicate that the Minto Flats is the reservoir for inconnu extending their range up the Tanana River. Until 1963 inconnu were seldom encountered in the Chena River (D. Evans, U.S. Fish and Wildlife Service, personal communication); during the past few years approximately 15/yr have been taken by sportsmen. No spawning populations have been located in the Chena River. In 1970 the first inconnu was reported from the mouth of the Salcha River, a Tanana River tributary, located 80 km upstream from the mouth of the Chena River. None of the tagged fish have been recovered farther downstream in the Tanana or Yukon River and it is felt that inconnu of this population are not esturarine anadromous.

The inconnu found in the tributaries of the upper Yukon River are also considered to be nonanadromous. No tagging studies have been conducted but they are slower growing than lower Yukon River inconnu (Alt 1973).

Management considerations — This tagging study has determined that fish harvested in upper reaches of rivers, e.g. Koyukuk, Chatanika, and Holitna, during upstream migrations and on summer feeding grounds belong to the same populations that are also subjected to harvest in the lower reaches of these river systems on overwintering grounds. Thus, an increase in harvest levels in one area would affect population levels at other locations along migration routes. I recommend that more detailed studies of population dynamics be undertaken before significant increases in harvest are contemplated.

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New Cell Line from the Marine Lumpfish, *Cyclopterus lumpus*[1]

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LI, M. F., AND S. CLYBURNE. 1977. New cell line from the marine lumpfish, *Cyclopterus lumpus*. J. Fish. Res. Board Can. 34: 134–139.

A cell line was grown from fin tissue of lumpfish (*Cyclopterus lumpus*). The cells were propagated in RTO medium supplemented with NaCl at 18°C and have a generation time of 108 h. They are fibroblast-like, contain numerous cytoplasmic granules, are susceptible to amphibian virus (LT-1), and have an average chromosome number of $2N = 50$.

LI, M. F., AND S. CLYBURNE. 1977. New cell line from the marine lumpfish, *Cyclopterus lumpus*. J. Fish. Res. Board Can. 34: 134–139.

Nous avons produit une nouvelle lignée de cellules provenant du tissue de la nageoire de la grosse poule de mer (*Cyclopterus lumpus*). Les cellules ont été cultivées dans un milieu RTO auquel on a ajouté un supplément de NaCl à 18 °C, et elles ont une période de génération de 108 h. Elles ressemblent à des fibroblastes, contiennent de nombreux granules cytoplasmiques, sont sensibles au virus amphibien (LT-1) et ont un nombre de chromosome moyen de $2N = 50$.

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THE in vitro culture of animal cells has proved over the past few decades to be a very important tool for disease and environmental pollution studies (Penso and Balducci 1963; Li et al. 1970; Li and Traxler 1972; Kruse and Patterson 1973; Li 1974; Li and Traxler 1974). Most studies on cultures from poikilothermic animals have dealt with freshwater fish, while information regarding cultures of marine fish tissue is rather limited (Clem et al. 1961; Townsley et al. 1963; Wolf and Quimby 1969; Lee and Loh 1975). Accordingly this report describes the growth, cellular morphology, viral susceptibility, and chromosomal studies of a cell line of fin tissue from the marine lumpfish (*Cyclopterus lumpus*) from the Canadian Atlantic coast.

Materials and methods — A mature lumpfish served as the donor for decontaminated fin tissue (Wolf and Quimby 1969). Explants were cultivated in RTO medium (Li and Stewart 1965) supplemented with 0.07 M NaCl (RTO–NaCl). Static cultures were prepared in Falcon tissue culture flasks (30 ml) with 2–3 ml of RTO–NaCl medium. The culture was maintained undisturbed at 18°C except for weekly medium changes. After 4 mo the cell sheets were confluent and were dispersed with cold EDTA–trypsin solution (Li and Stewart 1965) supplemented with 0.07 M NaCl for subculturing. The same procedure was followed to disperse monolayers for further subcultivation.

For growth studies the cells were grown in rubber-stoppered test tubes (16 × 125 mm) seeded with 2×10^5 cells/tube with 1 ml of the culture medium at 18°C. Three replicate tubes were removed at 2, 5, 8, and 11 days for growth enumeration with an electronic counter (Li and Stewart 1965; Li and Jordan 1969; Li 1974).

To study the viral susceptibility of the cultured lumpfish cells the virus (an amphibian virus, LT-1) (Clark et al. 1968) was propagated in fathead minnow (FHM) cells (Li and Traxler 1970). The lumpfish cell cultures were prepared in the test tubes or were grown on cover slips in Leighton tubes at 18°C. The 7-day old monolayer cultures were challenged with the appropriate virus dilution and incubated for 7 days at 20°C. At the end of the incubation period the cells in the test tubes were stained with Paragon-Formalin stain (Wheeler et al. 1968), and the cells grown on the cover slips were stained by the May-Grunwald and Giemsa method (Li 1974).

For chromosome preparation the culture medium of the 48-h cultures in the Leighton tubes was replaced by fresh medium containing 1 ug/ml of Colcemid (Ciba Co. Ltd., Dorval, Quebec). After 18–24 h the cells on the coverslips were subjected to hypotonic expansion by diluting the culture medium to one-quarter strength in cold distilled water for 2–2½ h at 18°C, and fixed in Carnoy's fixtative solution for 10 min; then the coverslips were air-dried, stained with 2% aceto-orcein for 15 min at 37°C, and mounted.

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FIG. 1. Cultivated lumpfish (*Cyclopterus lumpus*) fin cells showing fibroblastic appearance. A, 6-day-old culture (6th passage) — living cells under interference contrast; B, 4-day-old culture (4th passage) — May Grunwald-Giemsa stain. Bar = 50 μm.

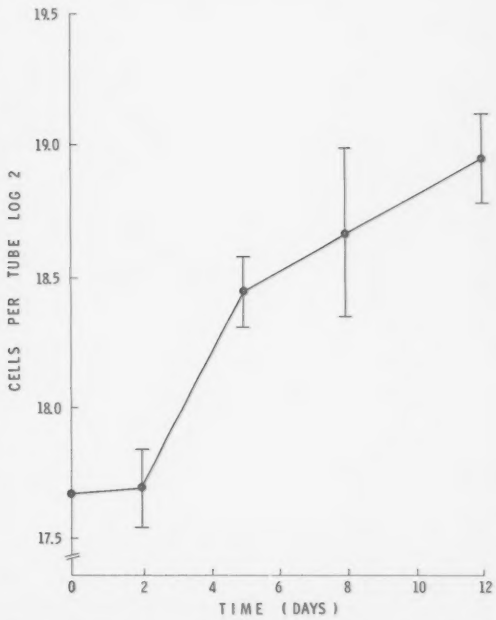

FIG. 2. Growth curve of the lumpfish cell culture in NaCl-supplemented RTO medium at 18°C. Vertical bars represent standard error of mean.

Results — Cell proliferation from the explants in RTO-NaCl medium took place after 5–6 days. An outgrowth around the explants was clearly noted after several weeks. The cells of culture outgrowth were harvested by cold trypsinization 4 mo after the initiation of the experiment. Newly seeded cells attached within 24 h and mitosis resumed by the 2nd day. Confluency of the cell sheet was achieved in 1–2 wk. Living cells and stained preparations of the cell cultures are shown in Fig. 1. The cells are mainly fibroblast-like. The mean growth rate of three replicate cultures is shown in Fig. 2. Mitosis appeared to begin 1–2 days after subcultivation; growth rate was most rapid between 2–5 days with a generation time of 108 h, and then decreased steadily.

Figure 3 shows control cells and those challenged with LT-1 virus. Control cells proliferated well for 7 days at 20°C (Fig. 3A), whereas those challenged with LT-1 virus ($10^{3.2}$TCID50 or $10^{4.2}$TCID50, where TCID = tissue culture infective dose) showed a pronounced cytopathic effect (CPE); some cells contracted and the structure of the whole monolayer culture was changed to the extent that some cellular destruction broke the cell sheet; a cytoplasmic inclusion-like body was

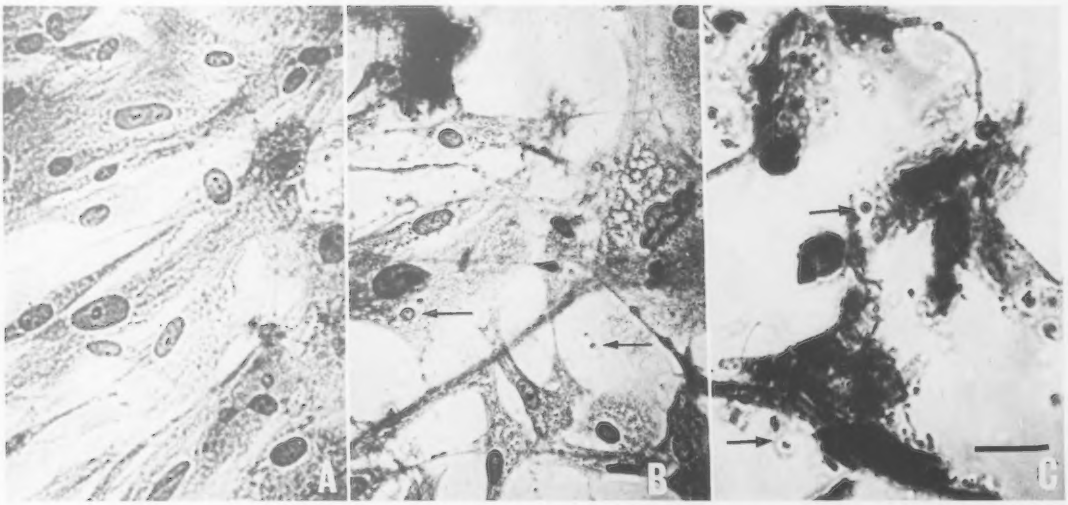

FIG. 3. Normal lumpfish cells and the cells infected with amphibian virus (LT-1), incubated for 7 days at 20°C. A, Normal cells; B, and C, cells challenged with virus ($10^{3.2}$TCID50 and $10^{4.2}$TCID50, respectively). The infected cells show cytopathic changes; large portions of the cells have been detached and the remaining cells are much altered; the presence of acidophilic inclusion-like bodies in the cytoplasm is marked by an arrow. May Grunwald-Giemsa stain. Bar = 50 μm.

frequently observed (Fig. 3B and 3C). The cytoplasm of the cultured cells contains numerous deeply stained particles (Fig. 4A) and these particles appeared to lose staining ability in the cells infected with the virus. Furthermore, a cytoplasmic inclusion-like body was frequently observed in the infected cells (Fig. 4B).

Chromosome counts per cell were performed in a number of metaphase plates (Fig. 5A) from several trials. The number ranged from 45 to 51 and the modal chromosome number was 50. Karyotyping of cells shows the presence of 4 pairs of submetacentric chromosomes, 6 pairs of subtelocentric chromosomes, and 15 pairs of chromosomes with a more or less terminal centromere (acrocentric chromosomes), of which one is marked by a peculiar long satellite extension (Fig. 5B).

Discussion — A number of primary cultures of marine fish from Canadian Atlantic waters have been described by Townsley et al. (1963). However, the present cell culture from lumpfish appears to be the first more extensive study of tissue culture from a marine fish caught from the colder waters of the Atlantic coast. Cells in continuous culture appeared to multiply more efficiently than those in primary culture. Though no plating efficiency data are available, a similar cell count in the inoculum and the count at 48 h may reflect that a high seeding efficiency was achieved (Fig. 2). The cells reached the logarithmic growth phase after a short lag phase and observed generation time is slighly longer than RTO cells cultured under similar conditions (Li and Stewart 1965). Many factors, such as initial cell density, incubation temperature, and nutrients in the culture medium, etc., have been known to have a significant effect on the growth rate of cultural cells (Li and Stewart 1965; Li and Jordan 1969; Plumb and Wolf 1971). The optimal conditions for this particular marine cell culture have not yet been determined.

The presence of massive cytoplasmic granules is unique to marine cell cultures. The infected cells appeared to show reduced granule staining properties; it would seem that the viral infection has resulted in an alteration of cellular metabolism or nutrient utilization. LT-1 virus produces cytoplasmic inclusion bodies in RTO cells (Li and Traxler 1970), and the nature of observed inclusion body-like particles (Fig. 3B, 3C) in the present culture is being further investigated. Unlike RTO or FHM cells (Li and Traxler 1970) the virus produces CPE in the marine cell. The variation of cytopathic pattern from one cell line to another under similar culturing conditions may indicate the specific interaction between the host cells and the infective agent (LT-1).

Though chromosome studies of various other

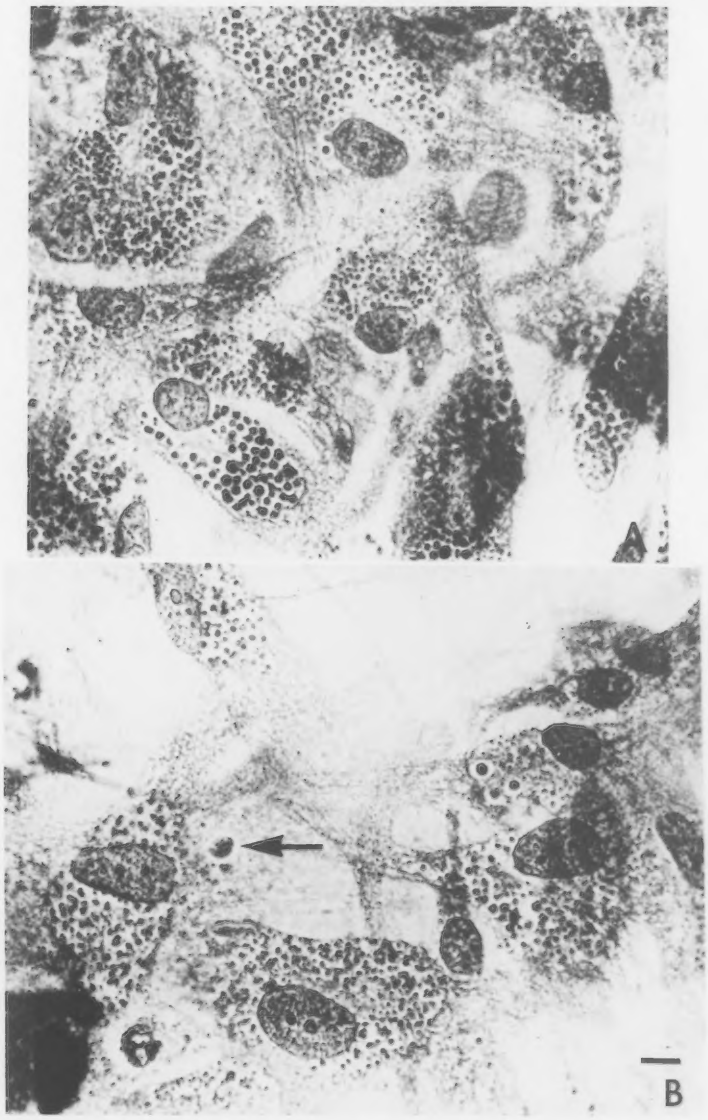

FIG. 4. Normal and infected (LT-1) lumpfish cells, incubated for 7 days at 20°C, at higher magnification. A, Normal cells; note the deeply stained cytoplasmic granules; B, infected cells ($10^{3.2}$TCID50); note the decrease in staining ability of the cytoplasmic granules. Arrow indicates inclusion-like bodies. May Grunwald-Giemsa stain. Bar = 10 μM.

fish have been well documented in the past few years (Roberts 1964, 1967; Regan et al. 1968; Chen 1969, 1970; Denton 1973; Lee and Loh 1975), a similar study on lumpfish has never been reported. The method of chromosome preparation described in the present study is reproducible in a number of trials. The coverslip–air-dry method is simple and does little damage to the hypotonically treated cells compared with the suspension and air-dry methods used by other

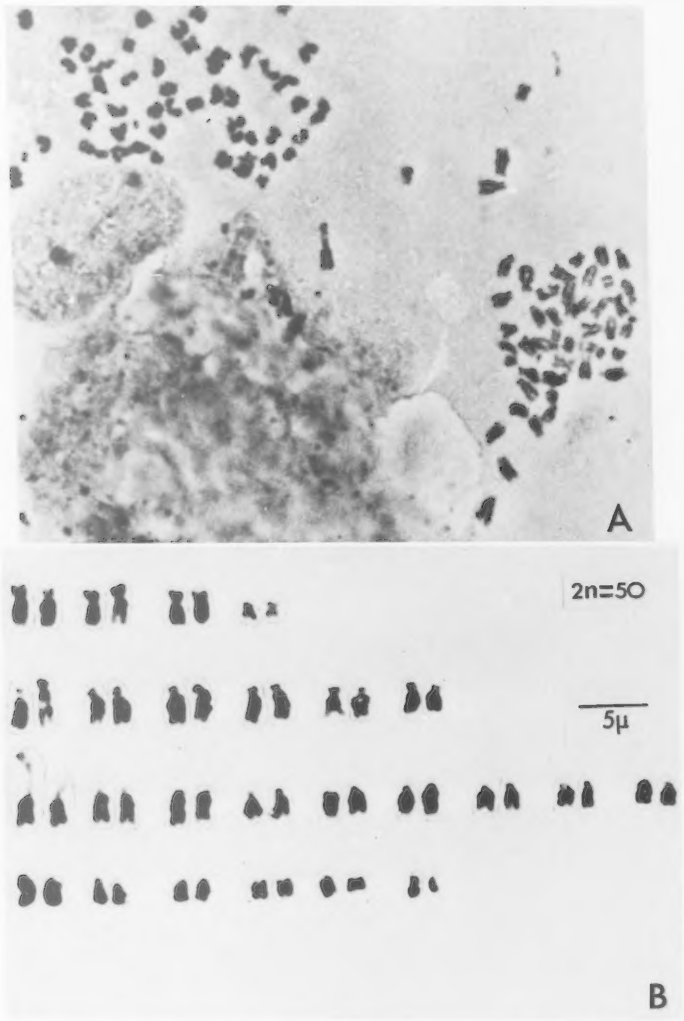

FIG. 5. Mitotic karyotypes in the lumpfish cell culture. A, Metaphase plates, showing chromosomes well dispersed; B, tentative pairing of the chromosomes. Note satellite chromosomes with long extensions on first pair of third row.

workers for other fish chromosome preparations (Regan et al. 1968; Chen 1970; Denton 1973). The peculiar satellite chromosome with its long extension could be a useful marker in characterizing specific karyotypes.

Development of epidermal papilloma in eel and neoplastic formation in marine fish appear to be closely related to environmental pollution (Peters 1975). Since chromosome breakage in humans could result from exposure to methylmercury (Skerfving et al. 1970) a study of chromosomal alteration of the fish cells exposed to pollutants would be of great interest. This marine fish cell line has reached the 18th passage and it is hoped that it will be useful for studying environmental pollution and marine fish diseases.

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Seasonal and Developmental Variation in the Lethality of Zinc to Juvenile Atlantic Salmon (*Salmo salar*)

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ZITKO, V., AND W. G. CARSON. 1977. Seasonal and developmental variation in the lethality of zinc to juvenile Atlantic salmon (*Salmo salar*). J. Fish. Res. Board Can. 34: 139–141.

The incipient lethal level (ILL) of zinc to juvenile Atlantic salmon (*Salmo salar*) in fresh water at a water hardness of 14 mg/ℓ varies from 150 to 1000 μg/ℓ as a function of season and developmental stage of the fish. The ILL increases from 500 to 1000 μg/ℓ during the 1st yr and decreases to 150 μg/ℓ in the following spring. The more sensitive stage in the salmon's life history, evidenced by decrease of ILL coincides with and is probably related to initial stages of the parr–smolt transformation.

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La teneur en zinc de début de mortalité (ILL) chez des jeunes saumons atlantiques (*Salmo salar*) dans de l'eau douce d'une dureté de 14 mg/ℓ varie de 150 à 1000 μg/ℓ, selon la saison et le stade de développement du poisson. La ILL augmente de 500 à 1000 μg/ℓ la 1re a. et diminue à 150 μg/ℓ au printemps suivant. Le stade le plus sensible du cycle biologique du saumon, tel que démontré par la diminution de ILL, coïncide avec et est probablement lié aux stades initiaux de la transformation tacon–smolt.

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THE lethality of zinc to juvenile Atlantic salmon (*Salmo salar*) has been extensively studied in connection with mining pollution of some salmon rivers in northeastern New Brunswick (see for example, Sprague 1964; Hodson and Sprague 1975). The incipient lethal level (ILL) of zinc in soft water (total hardness 14 mg/ℓ as calcium carbonate) is generally quoted as 500 μg/ℓ, and increases with hardness according to a relation established by Lloyd and Herbert (1962).

This paper demonstrates that the ILL of zinc changes with the season and size of the fish.

Materials and methods — Miramichi River stock, juvenile Atlantic salmon of the year, obtained from the Miramichi Hatchery, South Esk, New Brunswick, in September 1974, was divided into two groups and kept at ambient temperature (18–10°C). Starting at the beginning of November, water temperature for one group was maintained at approximately 10°C, whereas the other group of fish remained in water of ambient temperature (December–April, 4°C, followed by a slow increase to 15°C between May and July). The fish were fed Ewos dry food and kept under ambient photoperiod.

Toxicity tests were performed monthly from September 1974 to July 1975. Total water hardness was 14 mg/ℓ as calcium carbonate, and water temperature was the same as in the holding tanks, i.e. 10°C and ambient, respectively. Other experimental conditions were as described previously (20 ℓ of running water with 10 fish at each zinc concentration, duration 96–182 h, fish observed 2–3 times/day, Zitko et al. 1973). At least four concentrations of zinc were used in each test to determine the ILL (Sprague 1969) by plotting LT50 vs. zinc concentration in log–log coordinates (see for example, Sprague 1964). The concentration of zinc was determined by atomic absorption spectrophotometry in 2–3 samples/day and tank. Measured zinc concentrations were used in the data evaluation.

Results and discussion — The changes of zinc ILL from September to July are presented in Fig. 1. The ILL was increased initially and reached a maximum of approximately 1000 μg/ℓ in February. For fish kept at 10°C, the ILL then decreased to about 150 μg/ℓ in April and remained low for the duration of the experiment. The decrease of ILL was somewhat slower for fish kept and tested at ambient temperature. In this case the minimum ILL of 330 μg/ℓ was observed in June.

Seasonal changes of water hardness, alkalinity, dissolved nitrogen, and humic acid concentration were not detectable.

The tests by Hodson and Sprague (1975) on the lethality of zinc to salmon acclimated to different water temperatures were conducted in the fall and winter, i.e. before the onset of the period of increased sensitivity to zinc. The higher resistance of the fish to zinc in winter may be due to their lower metabolic rate during this period. The increased sensitivity to zinc in the spring may be caused by increased metabolic rate and physiological changes during the initial stages of the parr–smolt transformation. There is evidence that this transformation is size (Elson 1957) and photoperiod–pituitary (Komourdjian et al. 1976) related. For a recent review, see also Hoar (1976).

The size of the fish used in the toxicity tests and water temperatures during the tests are summarized in Table 1. The size of the fish at a given time of the year depends primarily on feeding intensity and water temperature. Consequently, the time of the onset of the increased sensitivity

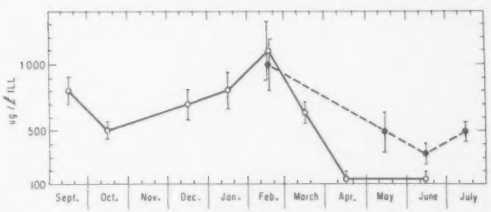

FIG. 1. Variation of incipient lethal level (ILL) of zinc to juvenile Atlantic salmon (*Salmo salar*). Solid and dotted line: fish kept at 10°C and at ambient temperature, respectively. Confidence intervals (95%) are indicated.

TABLE 1. Fish size and water temperature in toxicity tests.

Mo	Length (*cm*) Mean	Length (*cm*) Range	Weight (*g*) Mean	Weight (*g*) Range	Avg water temp (°*C*)
Sept.	5.3	4.7–6.1	1.6	0.8–2.6	14.7
Oct.	5.6	4.6–6.3	1.8	1.0–3.3	11.2
Dec.	6.2	5.3–8.1	2.8	1.5–6.2	9.8
Jan.	6.4	5.7–7.5	2.9	1.8–4.2	9.1
Feb.	6.9	5.3–8.7	3.6	1.6–6.0	8.7
	6.6	5.2–8.7	3.0	1.3–6.2	4.0[a]
Mar.	6.4	5.1–8.9	2.8	1.3–7.3	8.7
	10.0	8.7–13.8	13.8	7.0–26.0	10.4[b]
	9.0	7.5–12.5	7.3	5.1–10.5	8.9[a,b]
Apr.	7.1	5.4–9.8	4.0	1.6–10.8	9.7
May	6.2	5.6–8.2	2.7	1.3–6.0	7.2[a]
June	8.1	6.1–11.4	7.2	2.4–15.9	12.1
	7.3	5.6–8.8	4.5	1.5–7.0	14.4[a]
July	7.5	6.5–9.7	4.6	3.0–8.6	15.6[a]

[a]Fish kept at ambient temperature.
[b]1976 data.

to zinc may vary. For example, fish kept under identical conditions in 1975–76 and tested in March 1976 had ILL's of 140 and 500 $\mu g/\ell$ in the "10°C" and "ambient" group, respectively.

It is not known whether the change of juvenile Atlantic salmon's toxicant resistance is limited to zinc or whether it is of a general nature. There are some indications that the ILL's of copper and of the proposed reference toxicants, pentachlorophenol and dehydroabietic acid (Davis and Hoos 1975) do not vary as much as those of zinc. Additional data are needed to confirm this. The possibility that seasonal changes of sensitivity to toxicants occur with other anadromous species is worth exploring.

The current base-metal mine effluent regulations in New Brunswick are based on the resistance of 1-yr-old salmon parr to copper and zinc (0.1 toxic unit; see for example, Bell 1976). As demonstrated in this paper, the ILL of 2-yr-old juvenile salmon is approximately 4 times lower than that of the 1-yr-old fish. Therefore, the possibility of changing the effluent standards to encompass this sensitive life stage, the period from March to August, should be considered.

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Vital Statistics and Migratory Patterns of a Potamodromous Stock of Smallmouth Bass, *Micropterus dolomieui*[1]

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ROBBINS, W. H., AND H. R. MACCRIMMON. 1977. Vital statistics and migratory patterns of a potamodromous stock of smallmouth bass, *Micropterus dolomieui*. J. Fish. Res. Board Can. 34: 142–147.

Between 1968 and 1971, upriver movement of bass from Lake Simcoe was highly correlated with water temperature and discharge ($r = 0.704$, $P < .001$). Vital statistics and growth were similar to lacustrine and riverine stocks at similar latitude. Total annual mortality averaged 0.56 with 65% attributable to angler exploitation, principally in the river. Some 34% (after mortality) river spawners returned upriver in subsequent years. Repeat spawners (65% female) comprised an average 19% of the spawning runs. Most adults moved downriver in late June and July when water temperatures approached 25°C. Adults remaining in the spawning area experienced water temperatures to 29°C before emigrating during freshets in July and August. Lake migrants intermingled on shoal areas with lacustrine and possibly other potamodromous stocks each summer.

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De 1968 à 1971, les déplacements en aval de l'achigan à petite bouche du lac Simcoe ont été en étroite corrélation avec la température et le débit d'eau ($r = 0.704$, $P < .001$). Les statistiques biologiques et la croissance sont identiques à celles des stocks lacustres et fluviatiles de mêmes latitudes. La mortalité totale annuelle est de 0.56 en moyenne, 65% étant attribuables à la pêche à la ligne, principalement en rivière. Quelque 34% (calculé après mortalités) des individus qui fraient en rivière retournent en amont au cours des années subséquentes. Ces individus qui retournent ainsi frayer (65% de femelles) constituent en moyenne 19% des montaisons de fraie. La plupart des adultes se déplacent en aval à la fin de juin et en juillet, quand les températures de l'eau se rapprochent de 25°C. Les adultes qui demeurent dans la région de fraie sont exposés à des températures de 29°C avant d'émigrer au moment des crues de juillet et août. Les poissons migrateurs du lac se mélangent sue les hauts-fonds avec les stocks lacustres et possiblement potamodromes d'autres rivières à chaque été.

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ALTHOUGH there are numerous publications on lacustrine and riverine stocks of smallmouth bass, *Micropterus dolomieui* (e.g. Fraser 1955; Turner and MacCrimmon 1970; Paragamian and Coble 1975), published documentation of the existence of potamodromous stocks is limited to only two papers (Cuerrier 1943; Webster 1954), neither complete in several respects. The extent of potamodromy in centrarchid fishes is unknown but may be greater than is generally recognized. This paper reports on the vital statistics, river movements, and population structure of a Lake Simcoe stock which spawns in a 650 m length of the Pefferlaw River (44° 19′ N lat; 79° 13′ W long.) immediately below a mill dam 3.5 km from the lake; and is based on the examination of the total adult population reaching the upriver spawning grounds between 1968 and 1971.

Materials and methods — Movements of adult fish to and from the spawning area were monitored by 1.2-m nylon trapnet ([1.25 cm]² mesh) and a 0.9-m hoopnet [2.6 cm]² mesh) during May and June in 1968; and by a mechanical two-way weir from May 1 to October 15 in 1969, 1970, and 1971 (Ruggles 1974; Robbins 1975). The fence and trap were made of 0.96 cm iron rods spaced at 1.6 cm and captured all fish larger than 20.3 cm total length.

Routine data (Lagler 1956) were recorded on each fish which was then marked with a numbered Atkins tag (Rounsefell and Everhart 1953) attached just anterior to the first dorsal spiny-ray in 1967, and at

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the base of the first soft fin-ray in subsequent years. Tags on males were covered with orange, and on females with yellow fluorescent plastic tape for visual identification on the spawning grounds. As a check on loss of the Atkins tags, a jaw tag of monel metal was placed around the lower jaw of each fish in 1970. All fish were distinctively finclipped.

Efficiency of the downriver trap in a particular year was estimated by the proportion of fish tagged in the upriver trap and subsequently recaptured in the downriver trap in that year. The proportion of fish tagged or recaptured in the upriver trap and caught in the downriver trap the same year provided an estimate of the efficiency of the upriver trap. Population estimates based on the mark-recapture method were then determined (Robson and Regier 1968).

Scales, taken laterally from just posterior to the relaxed pectoral fin, were examined at 22.5 × and annuli determined (Creaser 1926; Reynolds 1965). As annulus formation occurred almost simultaneously with the spawning run, ages were interpreted as being 1 yr greater than the number of annuli unless the new annulus had just formed (Beckman 1942). As a check on the validity of the scale method for aging, the first pelvic spine was removed from a sample of 30 fish, sectioned, and examined at 36 × (Ostler 1975). Growth was estimated for both sexes by length-frequency distributions, length–weight, and age–length comparisons. Mean lengths of the various year-classes were determined by back calculation of individual fish using scale annuli (Everhart 1950).

Lake distribution of adults was monitored in Lake Simcoe each summer with trapnets and random creel surveys operated by the Ontario Ministry of Natural Resources, and by angler returns of fish tags.

A trap similar to that of Wolf (1950) was operated from July to October 1970, and May to July 1971 to determine the possible emigration of bass into the study area from a population resident above the mill dam.

River temperatures at the weir were monitored continuously, and river discharge daily. Analysis of the influence of river discharge and temperature on the intensity of the upriver migration run was made by regression from the 3 yr of data. Intensity of upriver migration on day d_o was related to the rate of discharge (cubic meters per second) on day $d_o - 1$ and to water temperature on day $d_o - 1$ at 1800 plus or minus the difference from day $d_o - 2$.

The annual mortality rate $\hat{a}$ was calculated from the following formula (Heincke 1913);

$$\hat{a} = \frac{X}{x + x_1 + x_2 + \text{-----}_n} \text{ where}$$

X is the frequency of occurrence of individuals at age X (the modal age class) and $x_1 + \text{-----} x_n$ is the frequency of occurrence of individuals in the subsequent age-classes. Results were then compared to the observed total mortality obtained from known fishing and natural mortality. Statistics with limits attached represent the "mean ± 95% confidence intervals" unless otherwise noted.

Results — Upriver movement — Upriver migration of mature adults began in early May, but no fish were captured in the weir until 2 wk after all lake ice had melted. A multiple partial correlation analysis revealed a high positive correlation between numbers of upriver migrants captured daily in the weir and river temperature and discharge levels ($r = 0.704$, $P < .001$). Between June 10 and 15, upriver migration typically ceased.

Statistics of spawning population — The number of ascending adults declined from 1969 through 1971 (Fig. 1, Table 1). No significant differences ($P > 0.05$) in the overall number of males and females ascending occurred, but more females than males were recorded entering the spawning area in 1971 ($P < 0.01$, $n = 638$).

Total length of male bass averaged 310.1 ± 2.4 mm with a modal class of 310.0–329.9 mm with corresponding average weights of 510.9 ± 14.8 g and modal class of 350–450 g. Females were significantly larger than males (*t*-test, $P < 0.05$), averaging 360.9 ± 2.3 mm with a modal class of 370.0–389.9 mm total length (TL), and average weight of 783.6 ± 18.2 g with modal classes of 750–950 g. All 50 (2% of total) immature fish were less than 260 mm. There was a significant annual decrease in fish size from 1968 through 1971. Total length equivalent in fork length was "0.946 × TL (mm) + 1.937." The length–weight relationship was "$\log_{10}$ weight (g) = − 2.094 + 3.177 $\log_{10}$ standard length (cm)."

Within the migrant population (Fig. 2), the

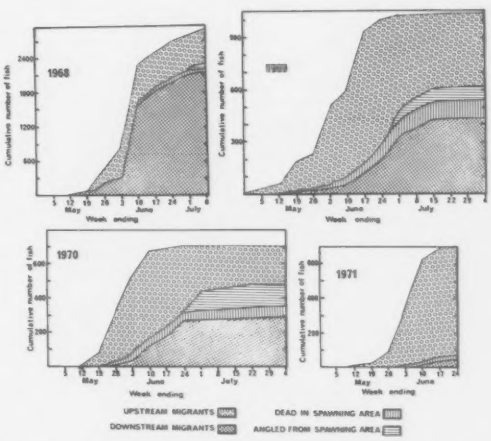

FIG. 1. Weekly accumulation of smallmouth bass in the spawning area, 1968–71 inclusive. Field studies terminated before the opening of the angling season, June 26, 1971.

TABLE 1. Estimates of the numbers of adult smallmouth bass (*Micropterus dolomieui*) in the upriver and downriver migrations, 1968–71.

	1968	1969	1970	1971
UP trap				
Trap efficiency (%)	28.7	73.1	94.8	100
Estimated number	2899 ± 337	1051 ± 24	707 ± 8	683
Down trap				
Trap efficiency (%)	7.8	75.4	73.1	—
Estimated number	2175 ± 182	459 ± 20	309 ± 14	—[b]
Natural mortality	53[a]	108	61	—[b]

[a]Incomplete count of dead fish because of initial net locations.
[b]Data incomplete; study terminated on completion of spawning.

dominant age-class in males was IV in 1969 and 1970, V in 1968 and 1971. Females of age V were most abundant each year. The oldest males were age IX and the oldest females age XII. About 50 immature fish of age-classes II and III migrated upriver in 1968 and 1969. Males entered the spawning area for the first time usually at age IV, females at age V. The 1963 year-class was dominant from 1968 through 1970; the 1966 year-class in 1971.

Estimated annual increments of growth, based on the relationship "scale radius (mm) = 0.031 × TL (mm) − 0.016," decreased from 102.9 mm during the 1st yr of life to 14.8 mm in the 11th yr. Calculated mean lengths were 103 ± 0.8 mm at age I, and 508 ± 20.0 mm at age XII (Table 2). Age estimates based on scale and finray methods were in agreement. Annulus formation was complete on 35% of the mid-May migrants and on 75% by the end of the run in mid-June. The sex ratio was 1:1, with larger and older fish typically reaching the spawning area first.

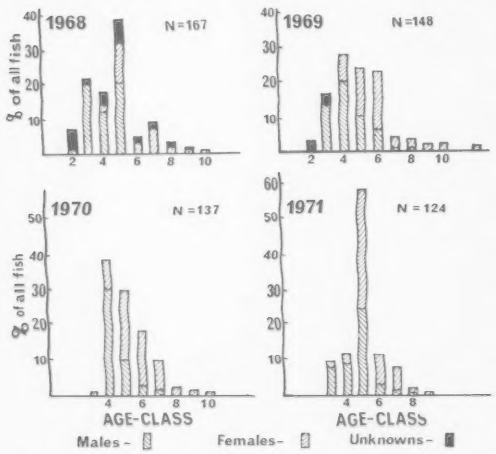

FIG. 2. Distribution by age-class of upriver migrants to the spawning area.

Mortality and repeat spawning — Total annual mortality, calculated from Heincke's formula (Heincke 1913), averaged 0.565 (0.466 for males and 0.484 for females) and closely estimated the observed mortality of 0.573 + .029 (Table 3). An intensive creel census in 1970 revealed that intensive legal and illegal angling in the spawning area resulted in a fishing mortality as high as 47 ± 2.9% of the spawning population. Most of the legal harvest occurred during the first part of the open season with an exploitation rate of 0.414 fish/rod h. Average natural mortality in the spawning area was 10% of migrant fish.

Repeat spawners comprised 18.8% of the migrants between 1969 and 1971, 61% of these female (Tables 1 and 4). An average 34.1% of surviving marked fish returned. Of those surviving fish marked in 1968, 31.7% returned once, 3.1% twice, and 0.2% in all three succeeding years.

Downriver movement and lake distribution — The principal downriver migration of adults occurred when mid-June water temperature reached 25°C, but a few fish remained in the spawning area through the summer months despite water temperatures to 29°C. Eventual emigration of all these fish was concurrent with heavy freshets (to 5.5 m^3/s). Between 43 and 70% of the upriver migrants returned downriver in the postspawning period. There was no appreciable difference in vital statistics between downriver and upriver migrants. Average loss of body weight while in the spawning area was 3.3% in males and 8.3% for females.

Fish returning to Lake Simcoe move generally northward to stony shoals at depths of 6 m or less where they intermingled with other river or lake spawning stocks. Intensive summer trapnetting, and random creel surveys indicated that

TABLE 2. Mean calculated total lengths (millimetres) of 1213 untagged smallmouth bass at end of each year life (sexes combined), Pefferlaw River, 1968–71. C.I. = confidence interval.

Age-group	No. fish	Year of Life 1	2	3	4	5	6	7	8	9	10	11	12
2	62	107.3	191.9										
3	344	104.1	182.5	255.1									
4	441	101.1	167.1	230.9	294.0								
5	136	99.6	153.3	204.8	262.2	317.1							
6	65	102.8	168.0	231.5	295.9	354.1	394.9						
7	65	106.4	168.7	231.1	291.1	342.8	386.6	418.8					
8	57	106.4	161.9	219.6	276.9	330.2	371.0	403.5	428.3				
9	27	107.9	169.8	225.0	283.7	332.1	373.2	406.0	432.8	450.1			
10	9	103.6	168.2	229.2	288.3	329.9	368.3	402.5	428.4	453.7	470.1		
11	2	92.5	145.9	202.3	267.8	324.1	371.7	398.5	419.2	439.9	451.8	463.5	
12	5	106.9	159.9	223.4	284.7	347.5	395.7	427.5	453.6	471.4	485.4	497.0	508.4
Mean length		102.9	171.0	234.3	286.8	332.4	382.9	410.6	430.6	452.8	472.6	487.4	508.4
95% C.I.		±00.8	±01.2	±01.6	±01.9	±02.8	±03.1	±03.2	±03.8	±07.7	±14.1	±22.1	±20.0
Number		1213	1213	1151	807	366	230	165	100	43	16	7	5
Annual increment		102.9	68.1	52.5	52.5	45.6	50.5	27.7	20.0	22.2	19.8	14.8	21.0
Males													
Mean length		102.2	172.1	236.7	285.1	318.4	380.4	409.2	433.6	458.1	495.7	514.4	527.0
95% C.I.		±01.0	±01.6	±02.0	±02.3	±05.6	±07.9	±07.2	±09.3	±19.1			
Number		715	715	708	426	100	36	25	14	6	1	1	1
Females													
Mean length		103.7	167.6	230.1	289.5	338.6	383.6	411.2	430.7	452.0	471.1	482.9	503.7
95% C.I.		±01.3	±02.1	±02.9	±03.2	±03.8	±03.5	±03.7	±04.5	±08.7	±14.7	±23.5	±20.9
Number		365	365	365	346	249	183	130	81	37	15	6	4

TABLE 3. Components of annual mortality observed in the potamodromous stock of smallmouth bass compared to estimates of total mortality obtained from the formula of Heincke (1913).

	1968	1969	1970	1971
Mortality estimated from Heincke's formula	0.66	0.31	0.39	0.69
Observed mortality				
In spawning area				
Recorded angler harvest	—	0.04± .002	0.08± .002	0.19± .003
Recorded natural mortality	—	0.02± .001	0.10± .003	0.09± .001
Unclassified mortality[a]	—	0.31± .024	0.39± .027	0.29± .023
In Lake Simcoe				
Recorded angler harvest	—	0.03± .002	0.07± .001	0.10± .001
Total observed	—	0.40± .029	0.64± .033	0.68± .027

[a]Magnitude determined from reduction in numbers of adults while in spawning area above weir.

TABLE 4. Number of returns to the spawning area from 1969 to 1971. Figures in brackets are percentages by sex. The figures in the table also include records of multiple recaptures in different years for 29 fish (see text for explanation).

Year recaptured		Year tagged 1968 Males	1968 Females	1969 Males	1969 Females	1970 Males	1970 Females	All years Males	All years Females	Both sexes
1969	Actual[a]	55 (35.2)	101 (64.8)					55 (35.2)	101 (64.8)	156
	Predicted[b]	56 (34.8)	105 (65.2)					56 (34.8)	105 (65.2)	161
1970	Actual	23 (46.0)	27 (54.0)	44 (46.3)	51 (53.7)			67 (46.2)	78 (53.8)	145
	Predicted	24 (47.0)	27 (53.0)	44 (44.9)	54 (55.1)			68 (45.6)	81 (54.4)	149
1971	Actual	7 (36.8)	12 (63.2)	20 (42.6)	27 (57.4)	27 (35.5)	49 (64.5)	54 (38.0)	88 (62.0)	142
	Predicted	7 (36.8)	12 (63.2)	20 (42.6)	27 (57.4)	27 (35.5)	49 (64.5)	54 (38.0)	88 (62.0)	142
All	Actual	85 (37.8)	140 (62.2)	64 (45.1)	78 (54.9)	27 (35.5)	49 (64.5)	176 (39.7)	267 (60.3)	443
	Predicted	87 (37.7)	144 (62.3)	64 (44.1)	81 (55.9)	27 (35.5)	49 (64.5)	178 (39.4)	274 (60.6)	452

[a]Actual number recorded returning to the spawning area.
[b]Estimated number of marked fish returning to the spawning area. This estimate is derived from the efficiency of capture of the upriver trap.

most fish remained within 4 km of the river mouth, but some traveled as far as 20 km.

Discussion — Because of the seasonal intermingling of Pefferlaw River fish with lacustrine stocks in Lake Simcoe, and a strong return (32–38%) of the spawners to the river, there is good evidence that this potamodromous stock is genetically isolated.

Upriver migration patterns of adult fish are remarkably similar to those reported for rainbow trout, *Salmo gairdneri* (Hartman 1959; Dodge and MacCrimmon 1971), and stress the importance of water temperature and discharge, and their interaction, in this migratory phase of potamodromy. While less precise, downriver emigration of spent bass is typically related to a water temperature of 25°C with summer freshets resulting in the exodus of stragglers from the spawning area. The resistance of these stragglers to high summer temperatures (to 29°C) is in accordance with the final laboratory preferendum of 28°C reported by Ferguson (1958), but the general emigration at lower temperatures is more in accord with observed relationships of smallmouth bass with temperatures in nature (Hile and Juday 1941; Hallam 1959). Webster (1954) attributed the earlier appearance of larger fish in riverine spawning areas, a phenomenon also observed in the present study, on lower temperature thresholds.

The suggestion of Webster (1954) that individual adults may not spawn every year in the riverine environment in strengthened by observations made in the Pefferlaw River in 1968 when traps located just downriver from the first riffle recaptured descending adults that had not entered the spawning area. More immature fish were taken at this more downriver location. Fluctuations in the size of spawning runs may be attributed principally to a combination of year-class strength and angling mortality.

Comparisons of the potamodromous population with riverine and lacustrine populations elsewhere reveals no significant difference in vital statistics. Growth and longevity (to age XII) is comparable to that in other north temperate waters (Fraser 1955; Latta 1963; Forney 1972; Paragamian and Coble 1975) and similar to lacustrine stocks in Lake Simcoe (Westman and Westman 1949). Good growth is attributed to an abundance of forage fishes and benthics (MacCrimmon and Skobe 1970). Females slightly outnumbered males in the spawning population ($P < 0.05$) with males predominant in younger age-classes and females after age V, reflecting differences in life span and a phenomenon also reported for Lake Ontario fish (Stone et al. 1954).

Mortality and exploitation rates can be compared only with lacustrine populations at northern latitudes. The average total annual mortality for the Pefferlaw spawning population (0.565) is higher than estimates of 0.232–0.602 for Cayuga Lake fish (Forney 1972) but similar to 0.58 for a Lake Michigan population (Latta 1963), although natural mortality (0.36) is far higher. Despite a probable elevation of angling pressure in the Pefferlaw River as a result of publicity given to the study, total annual mortality was not substantially greater than that reported for temperate lacustrine fisheries (Forney 1972; Latta 1963) because of a shift in balance from natural to angling mortality. Angling success in the river (0.42 fish/rod h) was similar to that of lakes Huron (0.51–0.87) and Michigan (0.25) but below that of Tadenac Lake (1.1) and Lake Opeongo (1.2–1.9) (Doan 1940; Fraser 1955; Latta 1963; Turner and MacCrimmon 1970). However, the inverse trend between size of spawning run and total mortality rates suggests that the maximum sustained yield of the fishery may have been exceeded.

Despite a lack of comparative data on potamodromy in smallmouth bass, it is apparent from this study that such populations are similar in vital statistics to temperate lacustrine populations of the species but, like the potamodromous salmonids, seasonal river aggregations of the spawning population offers a unique scope for scientific management especially population adjustment and river habitat improvement.

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Back-Calculated Fish Lengths and Hg and Zn Levels from Recent and 100-Yr-Old Cleithrum Bones from Atlantic Cod (*Gadus morhua*)

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SCOTT, J. S. 1977. Back-calculated fish lengths and Hg and Zn levels from recent and 100-yr-old cleithrum bones from Atlantic cod (*Gadus morhua*). J. Fish. Res. Board Can. 34: 147–150.

Dimensions of cleithrum bones from recently caught Atlantic cod (*Gadus morhua*) were measured and plotted against observed fish lengths to back-calculate cod lengths from cleithra from a ship wrecked in 1865. Mercury levels in the historical bones were approximately the same as those in recent material and showed no increase with fish length, but zinc levels appear to have increased since 1865 and increased with fish length.

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SCOTT, J. S. 1977. Back-calculated fish lengths and Hg and Zn levels from recent and 100-yr-old cleithrum bones from Atlantic cod (*Gadus morhua*). J. Fish. Res. Board Can. 34: 147–150.

Nous avons fait des mensurations des os de cleithrum de morues franches (*Gadus morhua*) récemment capturées et les avons portées sur graphique contre les longueurs observées de ces poissons afin d'obtenir par rétro-calculs les longueurs des morues à partir de cleithrums recueillis d'un navire qui avait fait naufrage en 1865. Les teneurs en mercure dans les anciens os sont à peu près les mêmes que dans le matériel récent et ne montrent pas d'augmentation avec la longueur des poissons, mais les concentrations de zinc semblent avoir augmenté depuis 1865 et augmentent en fonction de la longueur des poissons.

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IN April 1865, the steamer *Bertrand* sank in the Missouri River above Omaha, Nebraska. It was salvaged more than 100 yrs later in 1968–69. The cargo included a shipment of salt cod of which some boxes containing skeletal remains were retrieved. The cleithrum, part of the pectoral girdle, was the most conspicuous and measurable bone, 63 left and 79 right being recovered. The locality in which the cod were caught is not known but the shipment originated in Digby, Nova Scotia, so it may be assumed that the cod were from the Bay of Fundy or southwest Nova Scotia.

The Midwest Archaeological Center of the U.S. National Park Service was interested in determining the approximate lengths and weights of the cod in the shipment as a sidelight on the commercial and sociological practices of the period of the wreck. Five cleithra from the *Bertrand*, covering the size range of those recovered, were sent to St. Andrews for examination. Since no details of cleithrum to fish length relationships were available, cleithra, covering a size range which included that of the *Bertrand* material, were obtained from freshly caught cod of known length from the Bay of Fundy–southwest Nova Scotia area for comparison with the *Bertrand* material. At the same time advantage was taken of the opportunity to compare Hg and Zn levels in the *Bertrand* cleithra with those from the fresh material to see if there was a significant difference between the samples from the two periods.

Methods — For examination of recent material, cleithra were removed from the cod, cleaned, dried in air and, with the *Bertrand* material, measured as required to the nearest millimetre. Assays for Hg and Zn content were carried out by X-ray analysis on seven recent and five *Bertrand* cleithra.

Results and discussion — Cleithrum to fish length relationship — Cleithra were examined from 14 cod in the size range 26–131 cm (fork length). Four dimensions of each cleithrum (Fig. 1) were measured (not only total length as the tips of some of the *Bertrand* specimens were slightly damaged) and plotted against fish length (Fig. 2). The straight-line relationship between fish length and each of the four cleithrum measurements was defined by the intercept and slope from the simple regression y (cleithrum dimension) $= a$ (intercept) $+ b$ (slope) x (fish length, centimetres):

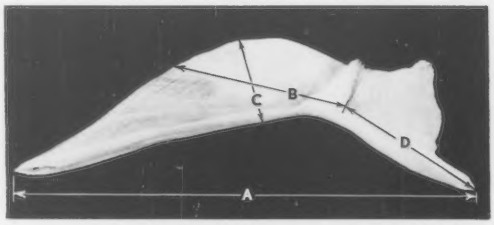

FIG. 1. Cod cleithrum showing the dimensions (A–D) measured for back-calculation of fish lengths.

Cleithrum dimension	a	b
A	–0.96	0.17
B	–0.43	0.07
C	–0.26	0.03
D	–0.39	0.06

The fish lengths represented by the *Bertrand* cleithra were estimated on the basis of these values (Table 1).

There was considerable variation in the back-calculated fish lengths derived from the different dimensions of each cleithrum. In particular, the A and D dimensions were low because of slight fraying and breakage, but A was the longest and, therefore, the preferred dimension; the left end (Fig. 1) of the B dimension was often difficult to locate accurately. However, the C dimension was the most reliable for accuracy but least preferred on the basis of its small size and the low slope of its regression; it gave unacceptably high back-calculated lengths for the largest cleithra in comparison to the lengths given by the other dimen-

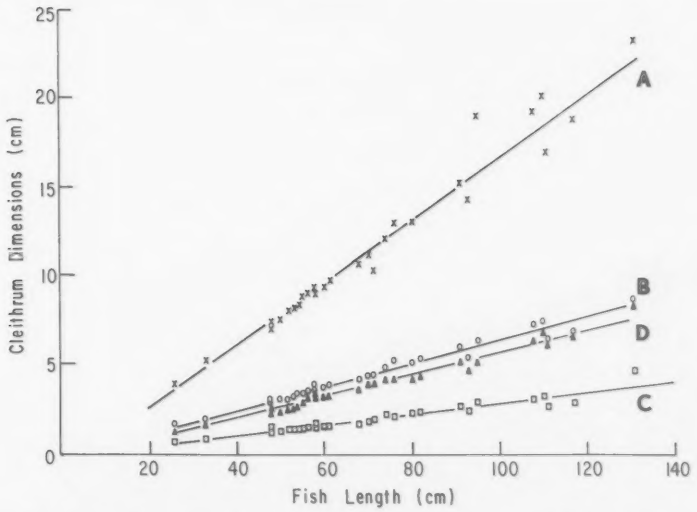

FIG. 2. Relationships between cleithrum dimensions and fish lengths in Atlantic cod (*Gadus morhua*). Dimensions A–D as in Fig. 1.

TABLE 1. Observed and back-calculated lengths of Atlantic cod (*Gadus morhua*) from cleithrum measurements A–D (Fig. 1). Material salvaged from *Bertrand*, 1–5; recent material from the Bay of Fundy–southwest Nova Scotia area, 6–19.

Fish number	Back-calculated fish lengths (*cm*)					
	Cleithrum dimension				Calculated length	Observed length
	A	B	C	D		
1	110	110	137	105	115	—
2	110	106	134	104	113	—
3	95	85	92	83	89	—
4	95	83	88	92	89	—
5	95	80	90	85	87	—
6	138	137	147	134	139	139
7	119	118	108	120	117	110
8	92	95	98	90	94	91
9	68	72	74	68	70	70
10	48	48	53	48	49	50
11	35	34	36	36	35	33
12	138	136	135	146	139	131
13	119	118	119	110	116	110
14	112	110	115	104	110	117
15	114	112	110	106	110	108
16	102	100	108	100	102	111
17	94	100	90	104	97	95
18	92	95	90	100	94	91
19	88	87	82	92	87	93

sions. A range of back-calculated values was derived from a selection of the recent cleithra and compared to the observed fish lengths (Table 1). Back-calculated fish lengths averaged about 2% above the observed fish lengths; no adjustment was made for this small difference. The mean of the back-calculated lengths from the four dimensions was taken to represent the back-calculated length of each cod.

The range of lengths calculated from the

TABLE 2. Mercury (Hg) and zinc (Zn) levels (ppb) in recent and 100-yr-old (*Bertrand*) cod cleithra in relation to fish length.

Area	Recent fish length (*cm*)	Hg	Zn	*Bertrand* fish length (*cm*)	Hg	Zn
Passamaquoddy Bay	58	60	42.5	81	50	38
	60	180	50.5	82	80	48
	71	80	48.0	83	65	37.5
	80	80	41.0	114	75	51.5
	82	80	50.5	117	75	50.5
Bay of Fundy and southwest Nova Scotia	91	5	59.0			
	93	20	59.0			
	95	45	58.0			
	108	65	58.0			
	111	17	53.0			
	117	30	61.0			
	131	5	61.0			
Mean	91.4	55.6	53.5	95.4	69.0	45.1

Bertrand material, 87–115 cm, corresponds to cod round weights of approximately 5.4–16.3 kg (Kohler et al. 1970).

Mercury and zinc levels — Mercury levels varied greatly in recent material — 5–180 ppb with no apparent correlation with fish length (Table 2). The fish 58–82 cm long were from Passamaquoddy Bay, and had a mean Hg level of 75 ppb, excluding one specimen with an aberrant value of 180 ppb. Fish of 91–131 cm (lengths not obtainable in Passamaquoddy Bay at the time of sampling) were from the Bay of Fundy and southwest Nova Scotia. They showed lowest (5 ppb) Hg levels in the smallest and longest fish, with a mean of 27 ppb. The Hg levels in the Bay of Fundy–southwest Nova Scotia samples were generally lower than those from Passamaquoddy Bay (Table 2) although they were from larger fish; higher levels would be expected if Hg were accumulated with size and age, assuming environmental Hg concentrations were similar in both areas.

The *Bertrand* material showed Hg levels at least as high as those of recent material (Table 2) except for the aberrant value mentioned previously. The greatest and least values in the *Bertrand* material were from fish of approximately the same length whereas the longest fish showed intermediate values. Mercury levels in recent fish of lengths comparable to the sizes of the *Bertrand* material were 80 ppb in 80–82-cm fish (*Bertrand* 65 ppb), and 17–65 ppb in 108–117-cm fish (*Bertrand* 75 ppb).

In general, Zn levels increased with fish length and were higher in recent material than for *Bertrand* material of comparable lengths. Levels in *Bertrand* material were 37.5–48.0 ppb in 81–83-cm cod (41.0–50.5 in recent fish), and 50.5–51.5 in 114–117-cm cod (53.0–61.0 in recent fish).

Sprague and Carson (1970) found 484 ppb Hg (wet weight) in bone from a 57-cm cod from Passamaquoddy Bay. The level in the bone was considerably higher than that in the liver (136 ppb) but near that of the muscle (428 ppb). Freeman et al. (1974) found 100–340 ppb Hg in muscle of Atlantic cod caught off Halifax Harbour. If Sprague and Carson's data, indicating that muscle and bone have similar Hg levels, are accepted, then the levels of Hg found in the recent and 100-yr-old cod lie well below the ranges found by Sprague and Carson and Freeman et al. Mercury levels in recent cleithra in the present study lie well below Freeman et al.'s (1974) upper levels, suggesting that there is considerable local variation in Hg concentrations.

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Critical Swimming Speeds of Coho Salmon (*Oncorhynchus kisutch*) Fry to Smolt Stages in Relation to Salinity and Temperature

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GLOVA, G. J., AND J. E. MCINERNEY. 1977. Critical swimming speeds of coho salmon (*Oncorhynchus kisutch*) fry to smolt stages in relation to salinity and temperature. J. Fish. Res. Board Can. 34: 151–154.

Critical swimming speeds (fork lengths per second L/s) of early fry, advanced fry, and pre-smolt coho salmon, *Oncorhynchus kisutch*, surpassed those of smolts. Performances were inversely related to size, varying from a peak of 7.3 L/s in fry to 5.5 L/s in smolts. In early fry to pre-smolt stages, salinity had little effect whereas in smolts a distinct maximum performance occurred near 13‰. Swimming performances of all stages varied directly with temperature: maxima occurred between 20 and 23°C. As temperature decreased, critical speeds of early fry declined at a faster rate than that of later stages, the values at 23 and 3°C being 7.2 and 3.5 L/s in early fry and 5.5–3.5 in smolts. This study shows that on a size-related basis underyearling coho are capable swimmers in estuarine conditions. Thus, their apparent failure to survive premature seaward migrations cannot be explained by their inability to perform important locomotor-dependent behaviors.

GLOVA, G. J., AND J. E. MCINERNEY. 1977. Critical swimming speeds of coho salmon (*Oncorhynchus kisutch*) fry to smolt stages in relation to salinity and temperature. J. Fish. Res. Board Can. 34: 151–154.

Les vitesses de nage critiques (longueur à la fourche par seconde, L/s) de jeunes alevins, d'alevins avancés et de présmolts de saumon coho, *Oncorhynchus kisutch*, sont supérieures à celles des smolts. Les performances sont en relation inverse de la taille, variant d'un sommet de 7.3 L/s chez les alevins à 5.5 L/s chez les smolts. Chez les jeunes alevins jusqu'aux stades de présmolt, la salinité a peu d'effet, alors que chez les smolts, il y a performance maximale distincte à environ 13‰. La performance de nage à tous les stades varie directement en fonction de la température : les maximums se situent entre 20 et 23°C. A mesure que la température baisse, les vitesses critiques des jeunes alevins diminuent à un taux plus rapide que celles des stades postérieurs, les valeurs à 23 et à 3°C étant de 7.2 et 3.5 L/s chez les jeunes alevins et de 5.5–3.5 chez les smolts. Cette étude démontre que, compte tenu de la taille, les saumons coho de moins d'un an sont de bons nageurs dans des conditions estuariennes. Leur apparente incapacité à survivre à des migrations prématurées vers la mer ne peut donc pas être expliquée par leur inaptitude à poursuivre d'importantes activités dépendant de la locomotion.

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IN some streams many coho salmon (*Oncorhynchus kisutch*) migrate to sea as underyearlings (Chapman 1962) in contrast to the normal life cycle where the 1st yr is spent in fresh water (Hart 1973). The ocean survival of premature migrants is apparently poor (Pritchard 1940; Foerster 1955; Milne 1962). The purpose of the present work was to test and compare the swimming abilities of coho early fry to smolt stages over a range of salinity–temperature combinations representative of freshwater (FW), estuarine, and inshore environments. Critical swimming speeds were measured in a stamina tunnel as an indirect assessment of their ability to perform important locomotor-dependent behaviors such as food capture, predator avoidance, and migratory swimming.

Materials and methods — Coho fry were collected from Cook Creek, Vancouver Island, during July and August. On transfer to circular, outdoor holding tanks at the Biological Station, Nanaimo, B.C., they were exposed to seasonal photoperiods and temperatures and a water velocity of approximately 1 fork length/s (L/s). Hand-feeding was begun with frozen brine shrimp (*Artemia salina*) and followed by a

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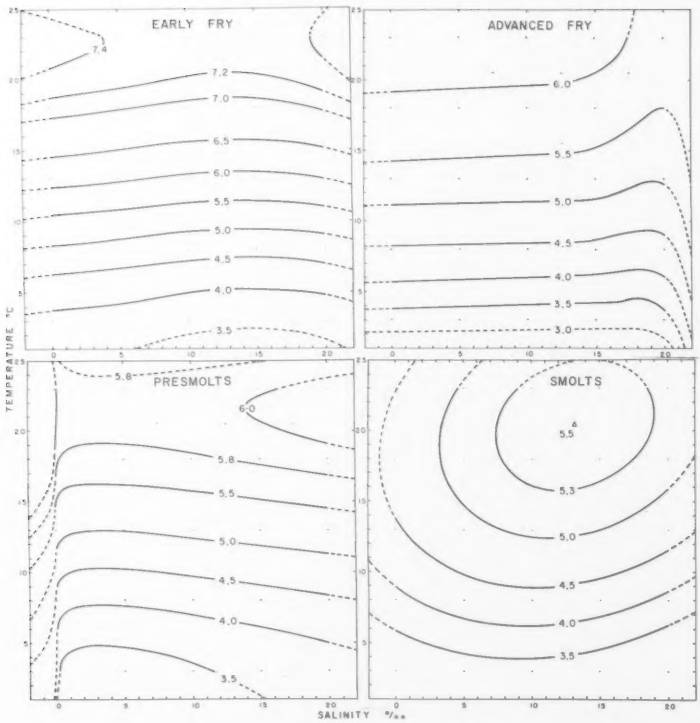

FIG. 1. Isopleths of critical swimming speeds (fork lengths per second) for juvenile coho salmon (*Oncorhynchus kisutch*). Test combinations are shown by (●); maximum critical speeds by (△).

regular diet of Abernathy pellets fed twice daily, 6 days a week.

The critical swimming speeds (Brett 1964) of early fry (July 29–October 1), advanced fry (November 16–December 30), pre-smolts (March 15–April 19), and smolts (May 27–June 23) were measured at a minimum of 25 salinity–temperature combinations (Fig. 1) in random sequence. Prior to testing each stage, fish were netted from the holding tanks, anesthetized with MS-222® (ethyl *m*-aminobenzoate methanesulfonate) and individuals of uniform length (mean population fork length ±1 SD) selected. Ten fish were then acclimated a minimum of 1 wk for each salinity–temperature test combination in 80 ℓ indoor tanks with changes from holding-tank conditions imposed as daily increments of 2.5‰ and 1.0°C (±0.5‰ and 0.1°C). Water velocities were adjusted with growth to 1 L/s. The photoperiod was maintained with fluorescent lights and a photocell switch.

Salinity–temperature combinations (±0.1‰ and 0.1°C) were established in a modified (Glova 1972) stamina tunnel (Griffiths and Alderdice 1972) at least 12 h prior to testing. For each test the 10 acclimated fish were transferred to the swimming apparatus and allowed a 1-h adjustment period with the water velocity at 1 L/s. The velocity was then increased by approximately one-eighth the estimated critical swimming speed at 1-h intervals (Brett 1964). Oxygen was maintained at near-saturation levels. Tunnel velocity was calibrated weekly with an Ott current meter. Ten fish per test and a 1-h adjustment period were selected as satisfactory on the basis of preliminary trials (Glova 1972).

Critical velocities were estimated by interpolation when fatigue did not coincide with the beginning or end of an hourly increment. Median critical swimming speeds were obtained from probits of individual values. All fish were returned to their acclimation tank for 24 h: no mortality occurred. Naive fish were used, except that approximately 80% of the smolts had already been tested once 4 mo earlier.

Performance surfaces showing isopleths of critical swimming speeds were constructed using a second-order nonlinear polynomial for early fry, advanced fry, and pre-smolts and a linear model for smolts (Alderdice 1971; Lindsey et al. 1970). In the case of smolts no improvement in the fit of the surface to the data resulted from using the nonlinear model. Analyses of variance were used to evaluate the relative importance of the linear, quadratic and interaction terms for salinity and temperature in the poly-

nomials. Although maximum likelihood ratios indicated that the salinity terms were of minor importance in early fry to pre-smolt stages, all the terms were retained since they represented the best available estimates of salinity–temperature effects.

Results — Swimming speeds for all stages of coho increased nearly linearly with temperature from 3 to around 20°C (Fig. 1). Critical velocities ranged from 7.3 L/s for early fry to 5.5 for smolts, results which compare favorably with those of Griffiths and Alderdice (1972). Salinity had much less effect on critical speeds than temperature (Fig. 1). Minor salinity effects were apparent in early and advanced fry responses. In both stages the configuration of the response surfaces suggests that ultimate critical speeds occur at salinities between FW and 12‰ and near the boundary temperature of 23°C. In pre-smolts, ultimate critical speeds also occurred near 23°C but at salinities near 15‰. Smolts showed an ultimate critical speed near 13‰ and 21°C. A significant salinity–temperature interaction (ANOVA $P < 0.05$) was also indicated: at low salinities maximum performance was associated with lower temperatures than at high salinities (low/low–high/high interaction: Alderdice 1971).

Assuming that variance was uniform over the field of test conditions, the estimated variability of swimming speed isopleths was ±3% for fry (based on fully replicated measurements at the 25 salinity–temperature combinations) and ±10% for smolts (based on replicated measurements at all points on both diagonals of the test space). This compares with a reported variation of ±7% for coho pre-smolts (Griffiths 1969). Prolonged culturing and reduced space with larger fish in the stamina tunnel probably contributed to increased variability with age.

Figure 2 illustrates the effect of size on swimming performance in relation to salinity and temperature. Relative critical speeds were inversely related to size, varying from a peak 7.3 L/s in early fry to 5.5 in smolts. This parallels findings by Brett (1965) and Bainbridge (1958). An interaction between temperature and size was also evident: slopes of the regressions progressively decreased and led to virtual extinction of size effects at 3°C. Younger fish were more severely affected (7.3–3.5 L/s) by decreasing temperatures (23–3°C) than were larger fish (5.5–3.5 L/s). Salinity effects were minor.

Discussion — This study demonstrates that relative critical swimming speeds (fork lengths per second) of underyearling coho salmon, with one exception, surpasses that of smolts. Underyearlings are less affected by salinity changes and

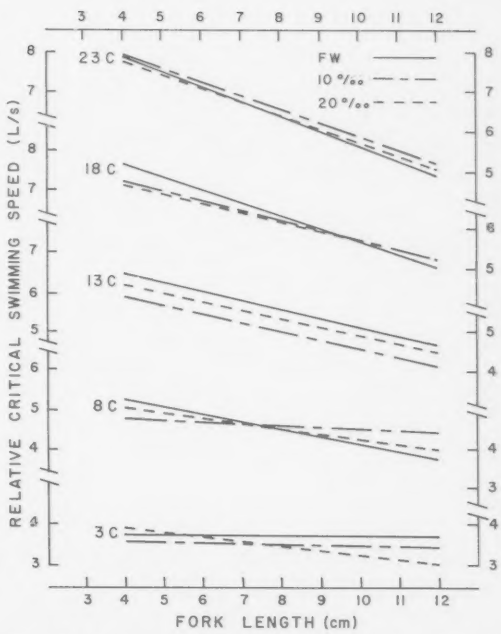

FIG. 2. Linear regression relations of median critical swimming speeds and fork lengths at three salinities and five temperatures.

their maximum critical velocities exceed those of smolts at all but very low temperatures. However, depressed swimming speeds in response to declining temperatures are more pronounced in fry than in smolts.

In the context of premature entry into the marine environment such relative comparisons could be misleading. For example, if predators of underyearling coho in the ocean are similar in size to those of smolts, then consideration of swimming speeds in absolute units (centimetres per second) would be important. Such absolute comparisons show fry to be much slower swimmers (Fig. 3a): the maximum performance of fry (36.1 cm/s) approaches the minimum performance of smolts (40.1 cm/s). This difference is underlined in Fig. 3b which shows that the chronological development of critical speeds is curvilinear, with fry falling 32% below what would be expected in a linear relationship.

A growing body of evidence shows that underyearling coho have the physiological capacity to regulate in salinities exceeding 20‰ provided a period of acclimation is allowed (Otto 1971; Conte et al. 1966). In addition, growth under laboratory conditions in hypertonic sea water is comparable to that in fresh water (Canagaratnam

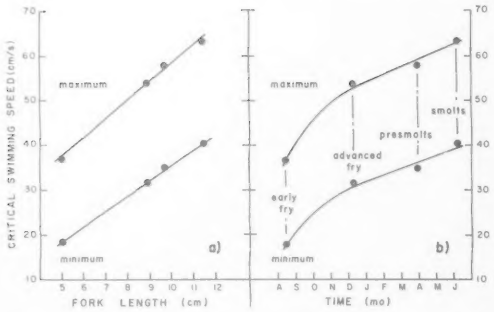

FIG. 3. The effect of: a, size, and b, chronological development on critical swimming speeds in juvenile coho salmon.

1959; Otto 1971). The present work shows that underyearling coho should be able to perform important locomotor-dependent behaviors in salinities up to 20‰. The anomalous situation in which large numbers of underyearling coho migrate prematurely to sea (Chapman 1962) but few if any survive (Foerster 1955) remains unexplained.

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Crayfish Growth in Lake Tahoe: Effects of Habitat Variation

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FLINT, R. W., AND C. R. GOLDMAN. 1977. Crayfish growth in Lake Tahoe: effects of habitat variation. J. Fish. Res. Board Can. 34: 155–159.

The natural history and ecology of the freshwater crayfish *Pacifastacus leniusculus* was investigated in Lake Tahoe for 1 yr. Growth of individual groups varied between three different transects (I, II, and III). The mean size of carapace of adult crayfish trapped on Transects II and III was greater than those trapped at Transect I. Linear regression analyses of premolt–postmolt carapace length revealed similar differences. At the onset of sexual maturity females from Transect I were smaller than those from either Transect II or III.

Examination of physical and biological parameters in the benthic habitat revealed that food availability, amount of shallow water, and size and area of stone cover influenced the number and size of adult crayfish on each transect. Greater food availability and more extensive stone cover on the lake bottom attracted higher numbers of crayfish to Transect I. The result was overcrowding and competition for food and larger stone cover for larger adults. The effect was a slower growth rate of crayfish on Transect I compared with animals on either of the other transects.

FLINT, R. W., AND C. R. GOLDMAN. 1977. Crayfish growth in Lake Tahoe: effects of habitat variation. J. Fish. Res. Board Can. 34: 155–159.

Les auteurs ont étudié l'histoire naturelle et l'écologie de lécrevisse d'eau douce, *Pacifastacus leniusculus*, dans le lac Tahoe durant un an. La croissance de groupes individuels varie à trois coupes transversales différentes (I, II et III). La taille moyenne de la carapace d'écrevisses adultes capturées aux coupes II et III est plus grande que celle d'individus capturés à la coupe I. Des analyses de régression linéaire de la longueur de carapace avant et après la mue révèlent des différences semblables. Au début de la maturité sexuelle, les femelles de la coupe I sont plus petites que celles des coupes II ou III.

Un examen des paramètres physiques et biologiques de l'habitat benthique démontre que la disponibilité de la nourriture, l'étendue d'eau peu profonde, et la taille et la superficie d'abris rocheux influent sur le nombre et la taille des écrevisses adultes de chaque coupe. Une plus grande quantité de nourriture disponible et un abri rocheux plus étendu sur le fond du lac attirent un plus grand nombre d'écrevisses à la coupe I. Il en résulte un surpeuplement et une concurrence à la fois pour la nourriture et pour l'abri rocheux plus étendu chez les grands adultes. Comme conséquence, le rythme de croissance des écrevisses de la coupe I est plus lent que celui des animaux à l'une ou l'autre coupe.

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INTERACTION between extrinsic environmental and biological factors affecting growth, survival, and reproduction determines the number and type of animals found in a given place. Hence, subpopulations, while occupying the same general habitat, may be living under completely different conditions because of environmental variation in portions of that habitat. In numerous studies (Van Deventer 1937; Momot 1967; Hopkins 1967) generically similar aquatic crustaceans living in different habitats have been shown to vary in growth and time to maturity. Differences in growth rates between local groups of *Pacifastacus leniusculus* in Lake Tahoe were suggested by Abrahamsson and Goldman (1970).

The freshwater crayfish *P. leniusculus* is the dominant benthic organism (by biomass) in Lake Tahoe and represents a very influential factor in the total lake dynamics (Abrahamsson and Goldman 1970; Flint 1975a). The growth rate of this species on Transects II and III was examined and reported (Flint 1975b). By describing the growth of *P. leniusculus* on a third transect of Lake

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Tahoe, comparing it with the previous transects, and discussing the effect of different local environments on this growth, this paper verifies that variations in growth rate do exist between groups of crayfish within the lake.

Materials and methods — Three transects showing variable bottom contour, bottom substrate, and productivity were selected on the west shore of Lake Tahoe, each positioned according to horizontal sextant angles taken simultaneously from visible marks on the shore. These transects extended from 5 to 100 m water depth. For the remainder of this manuscript, the transects will be referred to as I, II, and III, respectively (for detailed descriptions of each see Flint 1975a).

The techniques used to trap and mark the crayfish as well as the procedures for estimating growth were described previously (Flint 1975b). Momot (1967) showed that reliable estimates of population size could be made by the mark–repacture technique. In this investigation the Schnabel Method (Ricker 1958) was used to convert mark–recapture data into estimates of population size on each of the transects.

In situ estimates of crayfish density on each transect were made whenever possible. During the warmer months the use of SCUBA permitted 2-m^2 quadrats to be sampled (total of 67) at all three transects. Crayfish counts and sizes as well as the amount of available cover were recorded for each quadrat. In conjunction with the density estimates, surveys were conducted to determine the relationship between crayfish size and the size of the rock used for cover by the crayfish. We measured the perimeter of each rock providing cover and the size of the hidden crayfish.

Using probability paper and plotting cumulative percentage of ovigerous females against size-class (Wenner et al. 1974) we calculated estimates of mean size at onset of sexual maturity (ability to mate and extrude eggs). These data substantiated the effects of environmental parameters upon growth of different groups of the *P. leniusculus* population in Lake Tahoe.

Results and discussion — Abrahamsson and Goldman (1970) suggested that there may be large variations in the size of crayfish between locations in Lake Tahoe. Many environmental factors could cause this variation, and so three areas of the lake were investigated. The environmental differences between locations were then related to variation in crayfish growth.

Table 1 illustrates parameters that differed between the three areas. The number of adult crayfish estimated on Transect I was twice as large as at either of the other two sites. Transect I also comprised the most extensive area of shallow water, supported the highest density of crayfish, and displayed the smallest size of stones used for cover by crayfish. In addition, the boulder–cobble area was much more extensive on Transect I, due to the amount of shallow water. This in turn provided many more natural substrates for periphyton, the major food source of Tahoe crayfish (Flint and Goldman 1975).

The mean adult population carapace length obtained from trapping data over the duration of this study showed large variations between transects (Fig. 1). Transect II and III crayfish displayed similar mean lengths at most trapping intervals. Transect I animals, however, showed a mean carapace length that was at times as much as 10 mm below crayfish from the other two areas. Only during the October sampling periods did any similarity between all three locations occur. This deviation was due to the occurrence of intense mating activity (Flint 1975a) and seclusion of mature adults which allowed smaller organisms to become more numerous in the trap catches.

Molt increments were obtained from mark–recapture information. These were combined with laboratory molt data and in situ measurements (using SCUBA) of molt increases. Both items of information permitted us to construct linear regressions of premolt and postmolt sizes. Regression coefficients were calculated for male growth, female growth, and growth of crayfish on each of the three transects. A test of homogeneity of these coefficients showed that the only significant difference (0.5% level) was between growth of crayfish from Transects II and III compared with growth of crayfish on Transect I.

Using the length-frequency analyses of Tanaka (1962) mean carapace length for each age component from Transect I was estimated (Table 2) and compared with the estimated age components of Transects II and III (Flint 1975b). This comparison again indicated that there was a difference in crayfish length seen between the same age components of the different transects.

When comparing intraspecific groups or populations, one should have a convenient and objective measure of similarities or differences. Wenner's technique for determining mean size at onset of sexual maturity provided an acceptable method to assess differences between growth of crayfish on the three transects. This technique assumed that a population not affected by some environmental factor shows faster growth than a population living in a more "severe" environment. The faster growth, in turn, alters age and/or size at maturity (Wenner et al. 1974).

A probability plot of carapace length measurements obtained from all females trapped during the reproductive season in Lake Tahoe (Fig. 2) indicated the size at which 50% of the female crayfish were sexually mature. Linear plots for percent berried female crayfish on each transect were calculated with the least squares technique.

TABLE 1. Comparison of three transects studied in Lake Tahoe.

Transect and area studied[a] (m^2)	Estimated crayfish population (mark–recapture)	95% confidence limits	Mean density ($no./m^2$)	% shallow water[b]	Mean stone perimeter ± SE (cm)	Mean biomass (g/m^2)
Transect I 32,895.0	192,498	109,448–275,548	5.85	67.3	44.0± 13.18	11.695
Transect II 23,454.4	16,561	12,388–20,734	0.71	16.4	62.32± 14.17	1.729
Transect III 23,454.4	76,114	57,086–95,142	3.25	50.0	63.67± 16.28	5.820

[a]Based on mean collection radius for each trap of 21.5 m (Flint 1975a).
[b]Percent shallow water is the amount of bottom on each transect < 25 m deep.

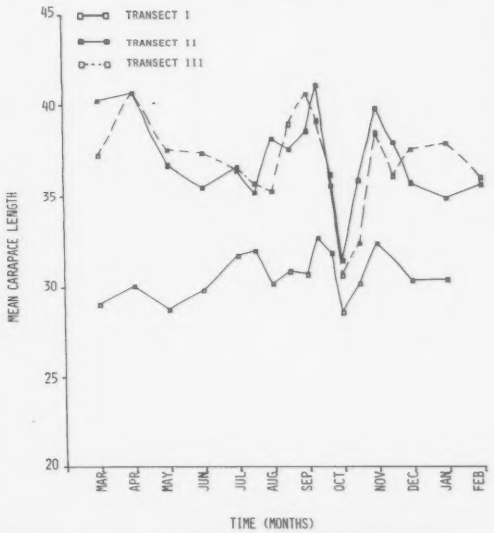

FIG. 1. Mean carapace length of crayfish (*Pacifastacus leniusculus*) caught in traps on the three transects in Lake Tahoe over the duration of this study.

The average minimum size of berried females on Transect I was smaller than average minimum size of ovigerous females on Transect II or Transect III. Furthermore, female crayfish from Transects II and III displayed the same mean minimum size at maturity. Presumably both groups were from the same population. Hence, the Transect I group of crayfish may have a different growth pattern than crayfish from the other two locations of the lake. This index does not permit precise error estimates or hypothesis testing. It does, however, quantitatively indicate the implications of the growth results (Fig. 1 and Table 2).

What accounts for the difference in growth of these local groups? We attempted to identify the more important environmental factors influencing

TABLE 2. Mean carapace length of age components in the crayfish (*Pacifastacus leniusculus*) populations from Transect I. Figures in parentheses indicate total number of crayfish in each sample. Starting point of each year-class assumed to be July. Size of the year-classes represented by mean carapace length (millimetres).

	Sampling period (1974)			
Year-class	July	Sept.	Oct.	June
0	3.50	7.25	8.00	12.00
I	13.00	17.00	16.50	19.50
II	20.50	25.00	25.50	27.00
III	28.50	30.50	30.50	31.00
IV	31.50	33.50	34.00	33.50
V	34.00	35.50	35.50	35.50
VI	35.50	36.50	36.50	37.00
VII	37.50	38.50	39.00	39.00
VIII	39.00	40.00	40.50	40.50
IX	40.50	42.00	42.50	42.00
X	42.00	45.00	44.50	44.00
XI	44.50	46.00	46.50	46.00
	(1353)	(465)	(1038)	(1195)

crayfish growth in the study sites. Temperature and light were similar between areas sampled. Analysis of stomach contents from more than 300 adult and juvenile crayfish collected from the three transects revealed no major difference in food selection between these locations (Flint 1975a). Numerous SCUBA observations characterized bottom in the shallower waters of each transect (Flint 1975a). Crayfish densities were directly related to the observed extent of stone cover, which varied on each transect. Additionally, size of stones available for cover and the percent of shallow water affected population numbers and also varied between transects. Crayfish in general avoided the colder deep waters during the growing and mating season (Flint 1975a).

Of the three locations, Transect II supported the smallest number of adult crayfish and the lowest biomass (Table 1). This location also had

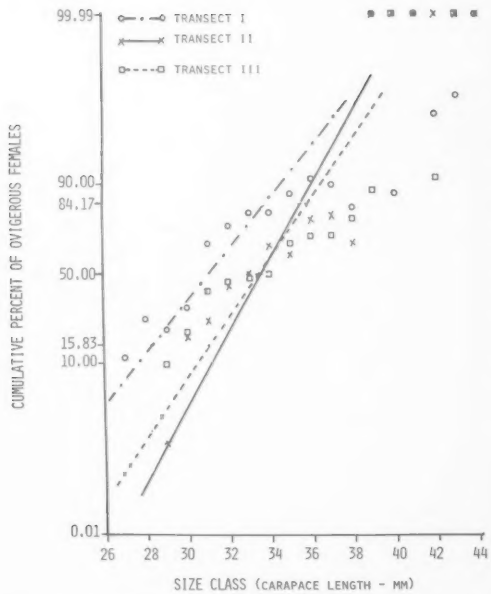

FIG. 2. Percent of ovigerous female *P. leniusculus* caught on each transect plotted on probability paper. This technique (Wenner et al. 1974) gives mean minimum size at sexual maturity for female crayfish as well as the standard error about the mean. A linear curve was fitted to each set of points for each transect using the least squares method.

the lowest percentage of shallow water and smallest area of boulder cover. Transect I, on the other hand, had the highest population number and biomass, largest percent of shallow water, and most extensive boulder–cobble coverage. The amount of boulder cover (stable substrate) was also directly associated with the periphyton growth on each transect. Consequently, Transect I supported more luxuriant growths of periphyton, the major food source of crayfish. This area of the lake was also shown to be more productive for periphyton (Goldman and Armstrong 1969; Flint and Goldman 1975) than other areas. These parameters appeared to be related at each transect. The direct relation between shallow water, area of stone cover, food availability, and population number (biomass), therefore, limited the number of animals maintaining themselves in any location of Tahoe.

The amount of lake bottom covered by cobbles and boulders, with their accompanying periphytic growth, was more extensive on Transect I. Consequently, the higher densities observed in this area may have been the result of recruitment from other areas or better survival of the young due to the presence of more food and cover. Thus, greater densities on Transect I lowered the growth rate because of crowding effects.

An additional factor affecting growth on the three transects was the relationship between size of crayfish and size of stone chosen for cover (Fig. 3). Crayfish are cryptic animals and in continual need of refuge against predation. Therefore, in Tahoe, they occupy crevices and burrows under rocks for cover. Crayfish accounted for 13–25% of the diet of lake (*Salvelinus namaycush*) and rainbow trout (*Salmo gairdneri*) (Frantz and Cordone 1970). During intermolt, crayfish cannibalized other individuals for up to 10.9% of their diet (Flint 1975a). A shortage of refuges for any period of time can be deleterious. High densities, therefore, require large amounts of appropriate-sized refuges. Since mean stone perimeter differed between locations (Table 1), there was a definite relation between the smaller crayfish on Transect I and the smaller boulders and cobbles. When the crayfish size classes were examined from the three areas, a major lag in growth was found in Transect I individuals after 20 mm carapace length (Table 2). According to Fig. 3, 20-mm crayfish usually selected a boulder in the size range of 40–50 cm. The mean size of

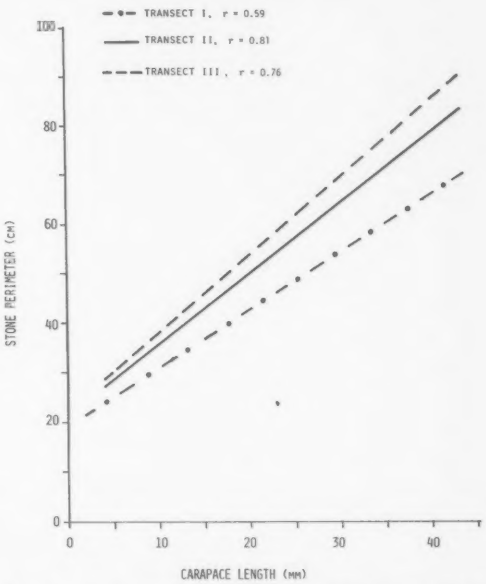

FIG. 3. The linear regression of individual crayfish size related to the size of the stone that it used for cover. Regression analysis performed for the three locations studied in Lake Tahoe. Correlation coefficients represent scatter of points about each curve.

boulders on Transect I, however, was 44 cm. Therefore, it appeared to be this stage in the life cycle of Transect I crayfish that growth was most affected by cover.

In conclusion, the crayfish population found on Transect I appear to be subjected to a number of factors not found on the other two transects. The high densities of crayfish in Transect I cause overcrowding resulting in competition for food and suitable cover which could cause a stunted population. The lack of suitable cover in conjunction with the crowding effects may act through time as a selection factor for individuals with slower growth rates. These conclusions agree with the speculation of Abrahamsson and Goldman (1970).

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Morphological Development of the Integument of the Sea Lamprey, *Petromyzon marinus*

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ANDERSON, W. C., AND P. J. MANION. 1977. Morphological development of the integument of the sea lamprey, *Petromyzon marinus*. J. Fish. Res. Board Can. 34: 159–163.

The thickness of the ventral body wall of the sea lamprey (*Petromyzon marinus*) increased 117% from June through January, and decreased 14% from January through May. During these respective periods, length increased 93% and decreased about 11%. Our studies suggest that the loss in length is part of the natural cycle; possibly prespawning adults cease feeding, or feed at a greatly reduced rate, beginning as early as January, rather than in March or April, as previously believed.

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L'épaisseur de la paroi ventrale du corps de la lamproie marine (*Petromyzon marinus*) augmente de 117% de juin à janvier inclusivement et diminue de 14% de janvier à mai inclusivement. Durant ces périodes respectives, la longueur augmente de 93% et diminue d'environ 11%. Nos études suggèrent que la diminution de longueur fait partie du cycle naturel; il est possible que les adultes au stade d'avant-ponte cessent de se nourrir ou se nourrissent à un taux beaucoup plus faible à commencer dès janvier, plutôt qu'en mars ou avril, comme on le croyait auparavant.

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ADULT sea lampreys (*Petromyzon marinus*) have been collected as part of the sea lamprey control program in the Great Lakes since 1953 (McLain et al. 1965). In 1969 the U.S. Fish and Wildlife Service began collecting parasitic-phase sea lampreys from the Great Lakes through payment of a bounty to commercial and sport fishermen (Smith and Braem 1972), to obtain specimens for studying seasonal changes in length and weight, maturation of the gonads, and sex ratio. One aspect noted in the lampreys examined was the variation in thickness of the body wall and integument in different months.

Information on the morphology and development of the integument of lampreys is limited, especially for those in the transformation and spawning phases of their life history. Published information on lamprey integument, the most recent by Downing and Novales (1971a–c) is restricted mainly to cellular composition of the skin and underlying tissues.

The present study describes the changes that take place in the morphology and development of the skin and underlying tissues of the sea lamprey from transformation to the spawning phase of its life history.

Materials and methods — Sea lampreys used in this study represent newly metamorphosed, parasitic, and prespawning phases of the life cycle. A recently metamorphosed lamprey was collected from a downstream trap in the Big Garlic River, where a known-age population was established in 1960 (Manion and McLain 1971). A total of 1417 parasitic-phase and prespawning lampreys were captured in lakes Superior and Michigan in commercial gear set for other species of fish during 1971. They were preserved in 10% formalin at the time of capture, and weighed, measured, and their sex determined at a later date. Thickness of the body wall was measured in 505 lampreys of this group captured in Lake Superior. To ensure uniformity and ease of measurement, we made the measurements (to the nearest 0.1 mm) at the mid-ventral region, posterior to the last branchiopore.

Six lampreys (one newly metamorphosed, four in the parasitic phase, and one prespawning adult) were used for histological studies of changes in body wall thickness. Sections of the body wall (5 mm^2) were removed from the middorsal line just posterior to the last branchiopore, embedded in paraffin, sectioned sagittaly at 6–8 μm, mounted on glass slides, and stained with hematoxylin and eosin. The sections were cut from the middorsal rather than the ventral region because the skin layers were thicker and cellular composition was more easily discernible.

The terminology used here agrees with that of Downing and Novales (1971a–c).

Results — Changes in thickness of the body wall — The ventral body wall became thicker as the lamprey increased in length (Table 1) and advanced from an early to a late parasitic phase. The thickness increased rapidly from June to October, and more slowly from October to January (Fig. 1). Average increases from June to January were 117% in body wall thickness and 93% in body length. The thickness of the body wall decreased gradually from January to March, and more sharply from March to April (Fig. 1). The total average decrease from January through May was 14% for the body wall and 11% for body length.

Histological changes in the skin — The epidermal layer of the lamprey integument is composed of club, granular, and mucous cells (Fig. 2A, B). The dermis has three distinct layers: a thick superficial subepidermal collagenous lamella, a thin layer of fibroblasts and melanophores, and a deep fat or adipose layer (Fig. 2A). Muscle is the principal underlying tissue.

The three cell types in the epidermis of newly metamorphosed lampreys are small and oval (Fig. 2A). The club cells appear to be limited to the basal region, and the granular cells are in the upper half of the epidermis, beneath the skin surface. The mucous cells, which are the most numerous, are interspersed between the basal cells and line the skin surface; they remain small and in this position through all life history stages.

The club cells in the parasitic-phase lampreys become elongated and extend from the basal area to about three fourths of the distance to the skin surface (Fig. 2B). Granular cells maintain about the same shape and position (upper half of the epidermal layer) as in recently transformed lampreys.

TABLE 1. Thickness of the ventral body wall of parasitic-phase sea lampreys of different lengths.

Total length (*mm*)		Number measured	Body wall thickness (*mm*)		
Range	Average		Average	Range	SD
<200	177	32	0.9	0.8–1.2	0.35
200–249	224	37	1.1	0.8–1.6	0.26
250–299	278	60	1.6	1.2–2.0	0.30
300–349	324	89	1.9	1.2–2.8	0.33
350–399	378	85	2.3	1.6–3.2	0.36
400–449	427	104	2.6	1.6–4.0	0.47
450–499	469	82	2.8	2.0–4.0	0.50
>500	516	16	3.2	2.4–4.4	1.35

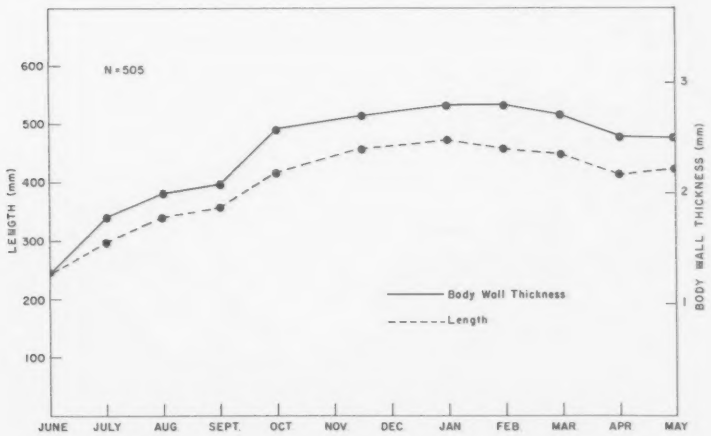


FIG. 1. Average length and thickness of the body wall of sea lampreys (*Petromyzon marinus*) from newly metamorphosed to the prespawning phase of their life history, Lake Superior, 1971.

Further changes take place in the cell types of spawning lampreys. The club cells shorten and granular cells appear to be interspersed throughout the skin (Fig. 2F). These changes are attributed to deterioration of the lamprey before its death, which occurs shortly after spawning.

The epidermal layer doubled in thickness as the lamprey passed from a newly transformed (Fig. 2A) to late parasitic (Fig. 2D, E) and prespawning phases (Fig. 2F). The most noticeable change in the dermal layer as a recently metamorphosed lamprey develops to a parasitic adult is the almost fourfold increase in the thickness of the subepidermal collagenous lamella and fivefold increase in thickness of the fat layer (Fig. 2A, E). The cytology of both layers between the transformation and parasitic phases differed little except that the horizontal orientation of the lamellae in the subepidermal layer became more noticeable as the lamprey increased in length (Fig. 2A, C). No differences were noted in the fibroblast–melanophore layer.

Although the thickness of the epidermis of the parasitic adult and the prespawning lamprey remained about the same, that of the dermal layer (except for the pigment layer) of the prespawning lamprey decreased. In the prespawning lamprey, the subepidermal layer had decreased 36% and the fat layer 60% of the thickness in the parasitic lamprey (Fig. 2E, F). After the parasitic-phase lamprey stops feeding, it evidently draws heavily on these two layers for subsistence and for maturation of the gonads.

Loss in length — Active feeding by lampreys is known to cease when they begin their spawning migration in the spring (Applegate 1950). Our data, based on the shrinkage of adult length from

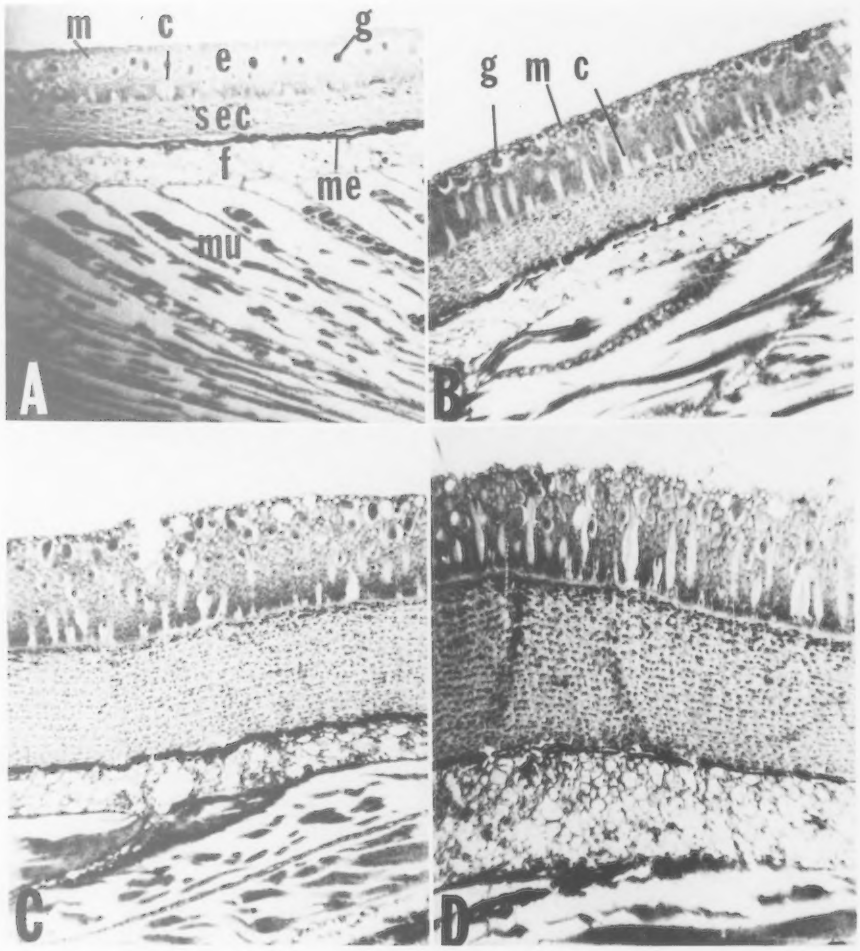
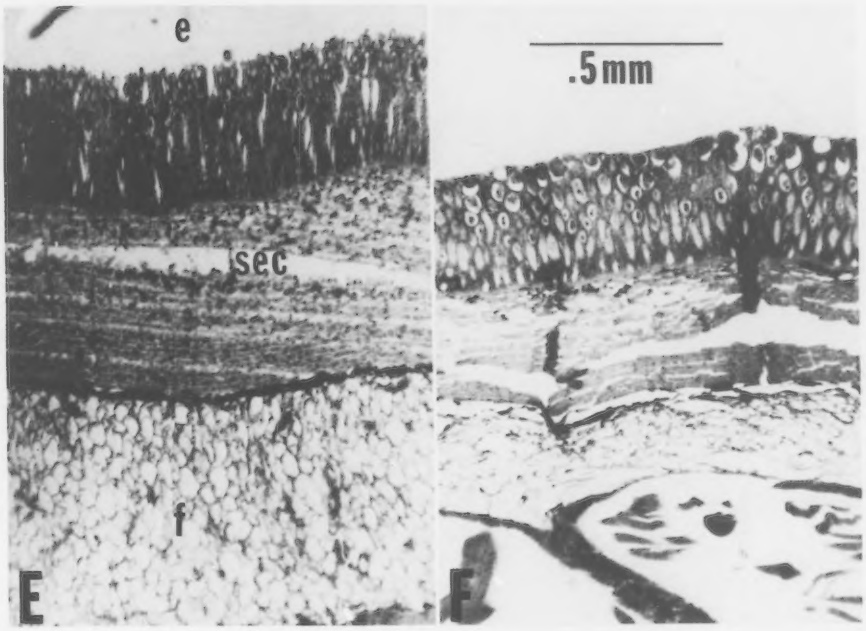


FIG. 2. Sagittal sections of skin of sea lampreys in different life stages: A, newly metamorphosed (160 mm long); B, parasitic phase in June (187 mm long); C, parasitic phase in July (235 mm long); D, parasitic phase in August (352 mm long); E, parasitic phase in December (500 mm long); F, prespawning phase (496 mm long). c = club cells; e = epidermis; f = fat; g = granular cells; m = mucous cells; me = melanophore; mu = muscle; sec = subepidermal collagenous lamella.

January to March, indicate that parasitic-phase sea lampreys may cease feeding or feed at a far reduced rate as early as January. The average loss of length in January–May was 11% for adults captured in Lake Superior (Fig. 1). Adults continue to lose length during the spawning season, as Manion and McLain (1971) found an average loss in length of about 14% (sexes combined) from early June through July. Thus lampreys apparently lose about 25% of their length from the time they cease feeding (perhaps in January) to completion of spawning (usually in July).

Moore et al. (1974), who mentioned the loss in length of tagged sea lampreys released in the Great Lakes basin, attributed this shrinkage (during a period when lampreys were believed to be actively feeding) to the paucity of large prey fish or adverse effects of the tags. Our studies indicate that some of this loss in length may also



be part of the natural life cycle, since lampreys apparently cease feeding earlier than was believed previously.

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Reliability of Laboratory Metabolic Measurements of Meiofauna[1]

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VERNBERG, W. B., B. C. COULL, AND D. D. JORGENSEN. 1977. Reliability of laboratory metabolic measurements of meiofauna. J. Fish. Res. Board Can. 34: 164–167.

The interstitial copepod *Hastigerella leptoderma* respires significantly less when sand grains are present than when sand grains are absent and the mud-dwelling species *Nannopus palustris* requires significantly different amounts of oxygen depending on the extraction method. Since the use of a substrate significantly reduced the metabolic rate of *H. leptoderma*, most meiofauna literature respiratory values are probably inflated estimates of the true metabolic rate. One must be careful how animals are extracted from the sediment because *N. palustris* extracted by differential gradient centrifugation respired significantly less than those hand sorted. Laboratory manipulation can significantly alter the estimated energy loss due to respiration.

VERNBERG, W. B., B. C. COULL, AND D. D. JORGENSEN. 1977. Reliability of laboratory metabolic measurements of meiofauna. J. Fish. Res. Board Can. 34: 164–167.

Un copépode interstitiel, *Hastigerella leptoderma*, respire nettement moins quand il y a des grains de sable que quand il n'y en a pas, et une espèce vivant dans la vase, *Nannopus palustris*, requiert des quantités significativement différentes d'oxygène selon la méthode d'extraction. Puisque l'utilisation du substrat abaisse notablement le taux métabolique de *H. leptoderma*, la plupart des valeurs respiratoires mentionnées dans la littérature sur la méiofaune sont probablement des estimations gonflées du taux métabolique réel. Il faut tenir compte de la façon dont les animaux sont extraits du sédiment, car les *N. palustris* extraits par centrifugation à gradient différentiel ont un taux respiratoire nettement inférieur à celui d'animaux triés à la main. La manipulation en laboratoire peut modifier la perte d'énergie estimée due à la respiration.

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THE recent interest in total benthic metabolism (Hargrave 1969, 1973; Pamatmat 1977) and particularly the role of meiofauna in total benthic energetics (Gerlach 1971; Vernberg and Coull 1974; Mills 1975) has spurred several researchers to measure the potential energetic loss to the system from meiofaunal respiration (Lasserre 1969; Coull and Vernberg 1970; Lasserre and Renaud-Mornant 1973; Schiemer 1973; Vernberg and Coull 1974; Hummon 1975). One of the questions that can be raised about any attempt to measure a rate function in the laboratory is whether or not the responses of the animals are similar to what they would be in the natural environment, and therefore whether the measured values are quantitatively valid. Such questions are particularly pertinent to meiofauna since collection, extraction, and sorting often involves techniques that require conditions the organisms do not usually encounter. Furthermore, since meiofauna live in close contact with the substrate, removal of the animal from the substrate could also have metabolic consequences. To gain some insight into the effects of such factors on metabolism, we measured the metabolic rates of the interstitial copepod *Hastigerella leptoderma* (Klie) with and without the presence of its normal sand substrate and of the intertidal burrowing copepod *Nannopus palustris* Brady following different methods of extraction.

Materials and methods — *Hastigerella leptoderma* and *N. palustris* were collected in the summer of 1975 from the North Inlet Estuary in Georgetown, South Carolina (33°12′N, 79°10.1′W), by using plastic buckets as scoops to collect the upper 2–3 cm of sediment. The buckets were filled to ⅓ their volume, capped, and transported to the laboratory in air-conditioned vehicles at a temperature approximating that of the field (25°C). Laboratory samples containing *H. leptoderma* were sieved through a 62-μm mesh and the *Hastigerella* sorted from the other meiofauna by mouth pipette and used experimentally within a few hours after collection.

We collected *N. palustris* in low intertidal muds,

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with large amounts of *Spartina* debris. Two primary extraction techniques were used to remove *N. palustris* from the muds: 1) the mud in the bucket was swirled by hand and decanted onto a 125-μm sieve; the material remaining on the sieve was then placed in finger bowls with sea water and *Nannopus* picked out with a mouth pipette and 2) by a combination of sieving and subsequent centrifugation and sucrose-flotation. This new modification of the standard sucrose–centrifugation extracting technique (e.g. Teal 1960) has been developed by W. B. Sikora of our laboratory. Since preliminary results indicated that centrifugation–sucrose flotation affected the metabolic rates, we then extracted *Nannopus* by sieving and either a) placed them in a sucrose solution without centrifugation or b) centrifuged them without sucrose. In all experiments the animals were used experimentally within an hour after extraction.

Respiration rates for both species were determined using Cartesian diver respirometers having a total volume of 9–11 $\mu\ell$ and filled with filtered 30‰ sea water, a salinity equivalent to that in the field. All determinations were made at 25°C (the field temperature) for approximately 1 h. After allowing 30 min for equilibration, readings were taken at 15-min intervals for a 1-h period. Oxygen consumption rates were essentially constant during time course of the determinations. One *H. leptoderma* (interstitial copepod) was used in each diver and used only once. The animals were run either in sea water without substrate or with a few grains of sand from the collection site. When run with sediment, a few sand grains were placed into the diver before the copepod was introduced. Determinations were made using both sterile sand and those in the nonsterile sand, results were pooled. A total of 12 determinations were made using sand and 12 using only sea water. A control diver containing sea water but no animal was used in each experiment; these blanks remained unchanged throughout the experiment, indicating that the system was functioning properly.

Oxygen uptake rates were measured for *N. palustris* following extraction by four different methods. In each series of experiments only one *Nannopus* was used per diver and no animal was used more than once. One group of animals was sieved and sorted without exposure to the sucrose solution or centrifugation. Eleven determinations were made on this group. Determinations were made on 13 animals that had been obtained using the centrifugation–sucrose flotation technique. Respiration rates also were determined on six animals sorted from the sucrose solution, having been exposed to it (without centrifugation) for 3 min. And finally, oxygen consumption was determined for six animals sorted from sediment by centrifugation but without sucrose. Under all experimental conditions the animals were checked at the end of the run to ensure that they were still alive and active.

Weights for both *H. leptoderma* and *N. palustris* were obtained by first killing the animals in 5% buffered formalin. The animals were then rinsed in distilled water to remove all traces of salt. Ten animals were placed on preweighed aluminum pans, dried to constant weight at 70°C for 24 h, and weighed on a Cahn electric microbalance (sensitive to ± 1 μg). Two to three replicate samples were used to determine mean dry weights for both species upon which the metabolic rates were based. Significant difference of means was analyzed using the Student t-test ($\sigma_1 \neq \sigma_2$).

Results and discussion — Data for *Hastigerella* are summarized in Table 1. Metabolic rates of *H. leptoderma* determined on animals without substrate were markedly higher ($\bar{x} = 53\%$) than the metabolic rates of those organisms where sand grains were provided as substrate and differences between mean rates were significant ($P < 0.05$). *Hastigerella leptoderma* is an interstitial copepod; it is vermiform and elongate. In the presence of a substrate, these animals grasp a particle of sand and either push themselves along slowly or curl around the particle and become relatively quiescent. In the absence of a substrate, the animals flail about vigorously; copepods do not become quiescent until immediately prior to death. This markedly elevated rate of activity in the absence of a substrate undoubtedly requires a much greater expenditure of energy which is reflected in the observed higher metabolic rate.

The results of our experimental work on interstitial *H. leptoderma* raises the following point of interest: metabolic rates measured for other truly interstitial taxa (e.g. oligochaetes, Lasserre 1969; mystacocarids, Lasserre and Renaud-Mornant 1971; gnathostomulids, Schiemer 1973; ciliates, Vernberg and Coull 1974; gastrotrichs, Hummon 1975) may be unusually high since none of those studies provided a sediment substrate for the experimental organisms. We suspect, however, that the presence or absence of a sediment substrate is not uniform in its effect on metabolic rate since the various taxa obviously use the sediment differently in their locomotion. Where interstitial harpacticoids grasp the particles during movement, gastrotrichs (cf. Hummon 1975) "glide" by ciliary motion and are therefore probably not as dependent on sediment as are those that use particles of substrate to aid in movement through sediment. Thus, in gastrotrichs the presence of sediment may not affect the respiratory rates as much as in the interstitial harpacticoids that grasp sediment during movement. Earlier studies have shown that metabolic rates can often be correlated with the habitat and hence the activity of the species (Coull and Vernberg 1970). Hummon (1975) in comparing his gastrotrich respiratory rates to other taxa states that the respiratory rates of *Turbanella ocellata* are "two or more times those for marine benthic . . . harpacticoids (Coull and Vernberg 1970). . . ." How-

TABLE 1. Oxygen uptake rates for *Hastigerella leptoderma* at 25°C with substrate provided and without substrate. Means are significantly different at $P < 0.05$ (*t*-test).

Condition	VO_2 ($\mu\ell\ O_2 \cdot h^{-1} \cdot$ mg dry wt^{-1})			
	$\bar{x}$	SD	SE	*n*
Water with sand grains present	4.90	1.97	0.57	12
Water alone	7.49	2.56	0.74	12

TABLE 2. Oxygen uptake rates for *Nannopus palustris* extracted by different techniques.

Extraction technique	VO_2 ($\mu\ell\ O_2 \cdot h^{-1} \cdot$ mg dry wt^{-1})			
	$\bar{X}$	SD	SE	*n*
Hand sorted	7.15	2.75	0.83	11
Sucrose and centrifugation	5.19	1.55	0.43	13
Sucrose, no centrifugation	6.76	2.01	0.82	6
No sucrose, centrifugation	6.30	1.18	0.54	6

ever, Hummon has compared his interstitial gastrotrich to our mud burrowing or epibenthic or pelagic species, and a comparison of the *Turbanella* values of 6.75–9.92 $\mu\ell\ O_2 \cdot h^{-1} \cdot$ mg dry wt^{-1} (converted to 20°C following Winberg 1971) to *Hastigerella* values without sediment (4.68 $\mu\ell\ O_2 \cdot h^{-1} \cdot$ mg dry wt^{-1} converted to 20°C) indicates that the values are higher but certainly not double or more. Hummon (1975) collected his experimental animals approximately 174 km north of where we colcolected the *H. leptoderma* discussed here but in similar sediments. Furthermore, since *T. ocellata* occurs at the sampling site where we collected *H. leptoderma* (E. E. Ruppert personal communication), we feel confident that they are somewhat similar in habitat preference and can thereby justify our comparison.

A major problem when dealing with meiofauna for experimental purposes is in procuring enough animals and efficiently extracting them from the substrate. Several methods have been developed for extracting animals from sandy substrates ($MgCl_2$, seawater ice, see Uhlig et al. 1973) but live extraction of meiofaunal species from mud presents a different problem. The sieving–centrifugation–sucrose flotation for extracting meiofauna from muds proved to be a very efficient method for obtaining large numbers of animals for experimentation. Although it was possible to extract large numbers of animals using this technique, the effects on their physiology were unknown. By measuring the metabolic rate of *N. palustris* extracted by various means were able to test the effects of centrifugation–sucrose on at least one meiobenthic mud species. The data indicate (Table 2) that the respiratory rates were only significantly lower where extracted by centrifugation in the presence of sugar ($P < 0.05$). Rates tended to be lower with other extraction methods but not significatly so.

Laboratory manipulation can have profound effects on metabolism. As Nichols (1975) has pointed out his respiration values for one heart urchin (*Brisaster latifrons*) accounted for more than 100% of the total measured in situ benthic metabolism. Did it react like our *H. leptoderma* almost doubling its respiratory rate in the absence of a substrate? Was it unduly "manipulated" in the laboratory giving highly inflated rates? Nichols, himself, has questioned the validity of his *Brisaster* respiratory data.

We present the foregoing to caution those attempting energetic models of the benthos based on laboratory respiratory values. Experimental conditions that allow suitable acclimation time to experimental conditions and minimize handling may provide more realistic estimates of the in situ metabolic rates.

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Species Diversity of Littoral Zone Fishes Along a Phosphorus–Production Gradient in Lake Memphremagog, Quebec–Vermont[1]

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NAKASHIMA, B. S., D. GASCON, AND W. C. LEGGETT. 1977. Species diversity of littoral zone fishes along a phosphorus–production gradient in Lake Memphremagog, Quebec–Vermont. J. Fish. Res. Board Can. 34: 167–170.

The species diversity of littoral zone fish communities in four areas of Lake Memphremagog was positively related to known phosphorus and primary production gradients along the north–south axis of the lake and to the associated north–south differences in macrobenthic and fish biomass. The highest diversity values were found consistently in the highly productive southern areas of the lake and the lowest values in the least productive northern areas.

NAKASHIMA, B. S., D. GASCON, AND W. C. LEGGETT. 1977. Species diversity of littoral zone fishes along a phosphorus–production gradient in Lake Memphremagog, Quebec–Vermont. J. Fish. Res. Board Can. 34: 167–170.

Nous démontrons une corrélation positive entre la diversité des espèces dans des communautés de poissons de la zone littorale dans quatre régions du lac Memphrémagog avec des gradients connus de phosphore et de production primaire le long de l'axe nord–sud du lac et avec les différences nord–sud qui leur sont associées dans la biomasse macrobenthique et celle des poissons. La plus grande diversité se rencontre invariablement dans les régions méridionales hautement productives du lac, et les valeurs les plus faibles dans les régions septentrionales moins productives.

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THE productivity of fresh waters is largely dependent on phosphorus input and availability (Schindler et al. 1973). Increased nutrient levels have produced marked changes in various trophic levels in experimental ponds (Hall et al. 1970) and have caused changes in the species diversity of freshwater phytoplankton communities (Moss 1973). These results suggest that changing nutrient levels and related changes in community productivity in natural environments may also cause alterations in fish species diversity. This has proved difficult to demonstrate because of the conflicting influences of exploitation, pollution, and other temporal changes in lake characteristics unrelated to phosphorus levels (review in Larkin and Northcote 1969).

Lake Memphremagog (45°06′N; 72°17′W) has a pronounced gradient in total phosphorus along its north–south axis (Kalff and Leggett 1976; Fig. 1) resulting from the combination of watershed and lake basin morphometry, and land use practices (Lands et al. 1974; Nakashima and Leggett 1975). Chlorophyll *a* is approximately 1.8 times (Ross and Kalff 1975) and macrobenthic biomass 3 times (Dermott 1974) higher in the south basin of the lake than in the north. The lake is not affected by industrial pollution. Its fish population has never been exploited commercially and recreational fishing is moderate in intensity. This combination of characteristics provides an excellent system in which to evaluate the influence of phosphorus levels on fish populations.

The purpose of this study was to determine whether fish species diversity is influenced by differences in levels of phosphorus and community production along the gradient.

Materials and methods — In 1974 and 1975 we used an 11.2-m bag seine (4.5 mm² mesh) to collect littoral zone fish species at 24 stations along the lake axis (Fig. 1). Collecting was done monthly from May to October between 0900 and 1700 h. Sampling stations were chosen to minimize differences in species diversity due to differences in local habitat.

It was not possible to obtain sufficient, unbiased, replicate samples at each station to calculate asymptotic values (Wilhm 1970). We therefore divided the lake into four regions representing four distinct levels in the phosphorus gradient (Fig. 1) and all samples taken in a single region within a 10-day period were pooled and considered to be replicates (Bechtel and Copeland 1970). This assumed some degree of between sample homogeneity, a valid assumption since asymptotic values generally occurred following pooling of three or more samples.

The diversity index

$$H = -\sum \frac{n_i}{N} \log_2 \frac{n_i}{N}$$

where N = total number of individuals in total population per area per unit time and n = total number of individuals of the ith species per area per unit time was used to calculate species diversity values for the littoral zone fish community. This approximation of the Shannon–Weaver formulation was chosen because it is independent of sample size at asymptotic values, emphasizes abundance among species, and is dimensionless (Wilhm and Dorris 1968).

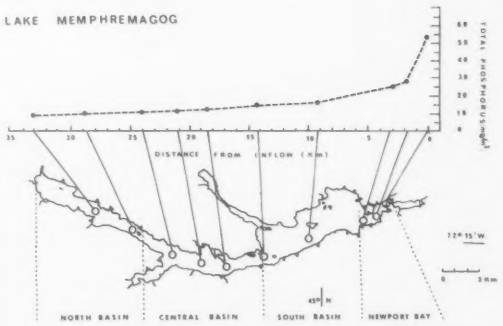

FIG. 1. Relationship between mean annual concentrations of total phosphorus (●– – –●) and distance along the north–south axis of Lake Memphremagog (from Kalff and Leggett 1976) and fish sampling locations. Closed triangles (▲) represent seine stations and open circles (○) represent total phosphorus sampling sites.

Diversity indices were based on abundance only. The data were separated into two groups: one comprised of adults of the permanent littoral zone fish community and a second composed of the total catch including the young-of-the-year. Significance of differences in species diversity between areas was tested with the Sign Test (Siegel 1956) since only the direction of the differences could be examined with reliability.

Results and discussion — Species diversity indices calculated for the permanent littoral zone fish communities of the two southern areas were higher than those for the central and northern areas (Table 1). The difference between Newport Bay (area 1) and the central (area 3) and north (area 4) basins was highly significant ($N = 15$, $x = 2$, $P = .004$) while the difference between the south basin (area 2) and areas 3 and 4 was less so ($N = 15$, $x = 4$, $P = .059$). This pattern closely mirrored the phosphorus–biomass gradient in the lake. The highest species diversity occurred in the region of maximum phosphorus concentrations and of maximum primary and secondary production, and the degree of statistical separation between areas declined as the phosphorus and biomass levels declined along the gradient.

During June and July of both 1974 and 1975 young-of-the-year of several species that do not utilize the littoral zone as adults were observed to

TABLE 1. Fish species diversity (H) values for littoral zone fish communities in four regions of Lake Memphremagog. A, permanent littoral zone species; B, total catch, young-of-the-year-included.

	Newport Bay		South basin		Central basin		North basin	
	A	B	A	B	A	B	A	B
1974								
June 21	2.03	2.12	2.34	2.24	.49	.38	.92	.38
July 30	1.49	1.91	2.33	1.00	1.27	1.54	1.23	1.35
Aug. 26	2.48	2.71	.55	1.36	.73	1.47	1.66	1.79
Oct. 6	2.73	1.89	1.92	1.97	.95	2.23	1.95	2.63
Mean	2.18	2.15	1.78	1.64	.86	1.40	1.44	1.54
1975								
May 22	1.53	1.52	2.13	2.13	1.46	1.44	1.49	.99
July 14	1.61	1.47	2.28	2.41	1.66	2.12	1.62	2.11
Aug. 30	2.44	2.37	2.40	2.97	2.21	2.28	2.17	.68
Oct. 22	2.47	1.54	.34	1.51			1.39	1.46
Mean	2.01	1.73	2.00	2.26	1.77	1.95	1.66	1.31
Grand mean	2.09	1.94	1.89	1.95	1.32	1.68	1.55	1.42

move into the area, possibly to feed or to avoid predators. This resulted in the occurrence of greater absolute numbers of species and pronounced changes in relative abundance of species in catches at all stations throughout the late spring and summer. This seasonal movement of young-of-the-year fish into the inshore area reduced, but did not obscure (Table 1), the general pattern of higher diversity in the more productive southern areas of the lake (area 1 vs. 3 and 4; $N = 15$, $x = 4$, $P = 0.059$; area 2 vs. 3 and 4; $N = 15$, $x = 6$, $P = 0.034$).

In addition to the pattern of species diversity noted above, Gascon (1976) found the distribution of two minnow species (*Notropis volucellus* and *Hybognathus nuchalis*) to be related to the nutrient gradient. The abundance of *H. nuchalis* declines steadily northward from the Newport Bay area because of reduced availability of detritus which is its principal food. The abundance of *N. volucellus*, however, which declines steadily southward from the north basin, is governed by levels of rooted aquatic vegetation which are highest in the phosphorus-rich southern basins. This species is known to reach maximum abundance in habitats having little rooted aquatic vegetation (Moyle 1973).

Thus the diversity of the littoral zone fish fauna of Lake Memphremagog is related positively to the nutrient gradient along its north–south axis and to the resulting north–south differences in primary production and macrobenthic biomass. Nakashima and Leggett (1975) and Gascon (1976) have shown that perch (*Perca flavescens*) and total minnow biomass are also positively related to the phosphorus–production gradient in Lake Memphremagog, being 2–3 times higher in the more productive southern basins. It thus appears that levels of phosphorus influence fish biomass and species composition in a manner similar to that previously reported for communities occupying lower trophic levels.

Acknowledgments — D. Levy, T. Cope-Arnold, J. Morris, D. Wong, and R. Leon assisted in the sampling program. Financial support for the Lake Memphremagog Project is provided by the National Research Council of Canada; Quebec Department of Education; Department of the Environment, Inland Waters Directorate; and the McGill Development Fund. Mrs T. H. Dunn and Mr J. Rutledge have generously provided space and facilities in support of the Projects' lakeside laboratories. OMC Corporation and Bombardier Inc. provided logistic support.

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COMING NEXT MONTH / À PARAÎTRE LE MOIS PROCHAIN

Editorial/Éditorial **J. C. Stevenson**

Changes in the size structure of cichlid populations of Lake Malawi resulting from bottom trawling **J. L. Turner**

Host–parasite relationships between sockeye salmon, *Oncorhynchus nerka*, and *Salmincola californiensis* (Copepoda: Lernaeopodidae) **Z. Kabata and B. Cousens**

Preferred, avoided, and lethal temperatures of fish during rising temperature conditions **D. S. Cherry, K. L. Dickson, J. Cairns Jr., and J. R. Stauffer**

Uptake from sea water and clearance of ^{14}C-*p*,*p*′-DDT by the marine copepod *Calanus finmarchicus* **G. C. H. Harding and W. P. Vass**

Population dynamics and marine mammal management policies **L. L. Eberhardt and D. B. Siniff**

Immunization of Pacific salmon: comparison of intraperitoneal injection and hyperosmotic infiltration of *Vibrio anguillarum* and *Aeromonas salmonicida* bacterins **R. Antipa and D. F. Amend**

Chemical and sensory changes in round inshore male capelin (*Mallotus villosus*) during prolonged storage for 24 months at −23°C **D. H. Shaw and J. R. Botta**

Heinz body hemolytic anemia in coho salmon (*Oncorhynchus kisutch*) exposed to chlorinated waste water **J. A. Buckley**

Surface area of the euphausiid *Thysanöessa raschii* and its relation to body length, weight, and respiration **G. C. H. Harding**

Life histories of *Cricotopus* (*Cricotopus*) *bicinctus* and *C.* (*C.*) *mackenziensis* (Diptera: Chironomidae) in the Fort Simpson area, Northwest Territories **D. M. Rosenberg, A. P. Wiens, and O. A. Sæther**

Responses to crude oil contamination by *Cricotopus* (*Cricotopus*) *bicinctus* and *C.* (*C.*) *mackenziensis* (Diptera: Chironomidae) in the Fort Simpson area, Northwest Territories **D. M. Rosenberg, A. P. Wiens, and O. A. Sæther**

Notes

Nutritional implications of surface feeding by gizzard shad (*Dorosoma cepedianum*) on insect remains in an Ohio reservoir **P. A. King, T. E. Wissing, E. J. DeVillez, M. J. Chimney, and W. B. Randall Jr.**

Factors in interpreting data obtained by diel sampling of fish stomachs **D. M. Eggers**

Rapid chromosome preparations from solid tissues of fishes **A. D. Kligerman and S. E. Bloom**

Comparison of an ecological model of Lake Ontario and phosphorus loading models **D. Scavia and S. C. Chapra**

Kinetics of lampricide (TFM, 3-trifluoromethyl-4-nitrophenol) residues in model stream communities **A. W. Maki and H. E. Johnson**

Occurrence of piscine erythrocytic necrosis (PEN) in the blood of the anadromous alewife, *Alosa pseudoharengus*, from Maine coastal streams **S. W. Sherburne**

Egg and larval survival of white suckers (*Catostomus commersoni*) at low pH **J. R. Trojnar**

Fecundity of *Balanus balanoides* (L.) in Passamaquoddy Bay **D. C. Arnold**

Fish orientation behavior: an electronic device for studying simultaneous responses to two variables **W. W. Reynolds**

Sex ratio of American eels, *Anguilla rostrata*, from the Matamek River system, Quebec, with remarks on problems in sexual identification **J. A. Dolan and G. Power**

Differences in swimming performances among strains of rainbow trout (*Salmo gairdneri*) **A. E. Thomas and M. J. Donahoo**

ANNOUNCEMENTS / AVIS

Call for Papers

11th Congress of the Canadian Meteorological Society

June 1–3, 1977
Winnipeg, Manitoba

The weather and climate of the plains area of Canada and the United States will be explored in the Congress theme "The Meteorology of the Great Plains." Sessions are also planned on physical meteorology, weather forecasting, micrometeorology, hydrometeorology, agricultural and forest meteorology, air pollution etc.

The Oceanography Division of the Society will be featuring the Canadian arctic with additional sessions on limnology, estuaries and coastal processes, and offshore oceanography. Contributions related to these areas are invited from all branches of oceanography and limnology. Contributions from biological, chemical, and geological oceanographers and limnologists are particularly encouraged. Invited speakers will present overview papers at the start of each session.

Titles and definitive abstracts of up to 300 words should reach the Program Committee by February 1, 1977. Meteorological papers should be sent to:

Mr Hugh M. Fraser
Atmospheric Environment Service
609 — 185 Carlton St.
Winnipeg, Manitoba
R3C 3J1

Oceanographic contributions should be addressed to:

Dr Noel Boston
Beak Consultants
602 — 1550 Alberni St.
Vancouver, British Columbia
V6G 1A5

The Congress will use the modern facilities of the Winnipeg Convention Centre with accommodation in convenient hotels. Further information will appear in the Society's journal *Atmosphere* or may be obtained from the Congress Coordinator:

Mr A. H. Lamont
Atmospheric Environment Service
600 — 185 Carlton St.
Winnipeg, Manitoba
R3C 3J1

Appel d'articles

11e Congrès de la Société canadienne de météorologie

1–3 juin 1977
Winnipeg, Manitoba

Sous le thème « La Météorologie des Grandes Plaines », le Congrès explorera la température et le climat des plaines du Canada et des Etats-Unis. Il y aura également des séances sur la météorologie physique, les prévisions météorologiques, la micrométéorologie, l'hydrométéorologie, la météorologie agricole et forestière, la pollution atmosphérique, etc.

La Division d'océanographie de la Société mettra en vedette l'Arctique canadien, avec des séances supplémentaires sur la limnologie, les estuaires et les processus côtiers, ainsi que sur l'océanographie des eaux du large. Toutes les branches de l'océanographie et de la limnologie sont invitées à présenter des contributions touchant ces domaines. Nous encourageons particulièrement les océanographes biologistes, chimistes et géologues à présenter des travaux. Au début de chaque séance, des orateurs invités donneront une vue d'ensemble du sujet.

Les titres et les résumés définitifs, ne dépassant pas 300 mots, devront être transmis au Comité du programme avant le 1er février 1977. Les articles portant sur la météorologie devront être adressés à :

M. Hugh M. Fraser
Service de l'environnement atmosphérique
600 — 185 rue Carlton
Winnipeg, Manitoba
R3C 3J1

Les contributions portant sur l'océanographie devront être adressés à :

M. Noel Boston
Beak Consultants
602 — 1650 rue Alberni
Vancouver, Colombie-Britannique
V6G 1A5

Le Congrès utilisera les installations modernes du Centre des conventions de Winnipeg, avec accomodations dans des hôtels à proximité. De plus amples renseignements apparaîtront dans le périodique de la Société, « *Atmosphère* », ou peuvent être obtenus du coordonnateur du Congrès :

M. A. H. Lamont
Service de l'environnement atmosphérique
600 — 185 rue Carlton
Winnipeg, Manitoba
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Journal of the Fisheries Research Board of Canada

Guide for Authors

A guide on preparation of manuscripts from theses is also available from the *Journal*.

Content

We welcome manuscripts reporting significant new information in the broad fields of fisheries management and technology, ocean science, and aquatic environments relevant to Canada. Manuscripts must not have been submitted or accepted for primary publication elsewhere. In general, manuscripts reporting fragments of research are unacceptable, as are those of mainly local interest.

Articles: Studies of broad scope that are original contributions to science.

Notes: Differ from articles on the basis of scope or completeness, rather than quality of research. They may report significant new knowledge arising from problems with narrow, readily defined limits, or important findings that warrant immediate publication before broader studies are complete. Notes should occupy no more than 3 printed (9 manuscript) pages.

Reviews: Detailed critical appraisals of broad areas of investigation, particularly those that have developed rapidly in recent years.

Perspectives: Essays of opinion or hypothesis on aquatic science that are of concern to the professional and layman. References are desirable; informal style is welcomed.

Letters and Comments: Opinion on topics recently discussed in the *Journal*; preferred maximum length, 500 words.

Editorial Practices

The *CBE Style Manual* (3rd ed. AIBS, Washington, D.C. 20016) is used as the authority in matters of form. The standards for spelling are: *Webster's Third New International Dictionary* and *le Grand Larousse Encyclopédique*. Titles of periodicals are abbreviated as in the *Biological Abstracts* list. References to literature are made by the name-and-year system. The *Journal* follows the names and spelling recommended in *A List of Common and Scientific Names of Fishes from the United States and Canada* (3rd ed. 1970) Spec. Publ. No. 6, American Fisheries Society. All measurements must be metric and SI units are preferred.

Series titles should be avoided. An *abstract* is required for all manuscripts, and should state concisely, in less than 250 words, what was done, found, and concluded. Up to 10 *key words* should follow the abstract.

Each manuscript is normally submitted to two *referees*. Contributors are encouraged to suggest names of potential referees.

Illustrations should be submitted in triplicate as photographic reproductions. They should not be mounted, stapled, or clipped. Symbols, letters, and numerals must be neat, and at least 1.5 mm high when the illustration is reduced to column width. Drawings should, when possible, be grouped to facilitate comparisons.

Proofs are sent to the author, who is responsible for correcting errors in typesetting. *Alterations to the content must be avoided.*

Fifty *reprints* are supplied free. Additional reprints may be purchased at the time of publication (an order form is sent with the proofs). A charge of $40.00 per page is made for articles published. When submitting a manuscript, an author should state that his institution will accept this charge.

Checklist on Manuscripts

Attention to the following questions will expedite appraisal of manuscripts by referees and editors.

Are the findings, interpretations, and conclusions adequately documented and relevant to the purpose of the study?

Are all the tables and figures needed, and organized to facilitate comparisons? Are there inconsistencies between tables or figures and the text, or within the text? Should some of the data be made available separately, in a data or manuscript report or at a data repository? If you use statistical analysis are the statistics subordinate to the research?

Would any of the text be clearer if condensed? Are summary statements given at the beginning of sections and paragraphs, and are details in sections and paragraphs relevant to their topics? Does the organization of the manuscript follow logically from the statement of purpose in the introduction?

Does the *title* encompass the content of the report? Does the *abstract* give the essentials of the new knowledge? Is the *introduction* largely limited to the scope, purpose, and rationale of the study? Is review of the literature limited to defining the problem? Are details of *materials and methods* limited to what scientists need in understanding the design of the study and in judging adequacy of the data? Are generalizations on the *results* adequate? Are findings distinguished from inferences from them? Is *discussion* limited to interpretation and significance of the findings?

Journal de l'office des recherches sur les pêcheries du Canada

Guide des Auteurs

On peut se procurer également auprès du *Journal* un guide pour la préparation de manuscrits à partir de thèses.

Sujet

Les manuscrits comportant une information nouvelle et significative dans le vaste domaine de la gestion et de la technologie des pêches, des sciences de la mer et des milieux aquatiques au Canada sont les bienvenus. Ils ne devront pas avoir été soumis ou acceptés pour publication dans d'autres périodiques de fonds. En général, les manuscrits portant sur des fragments de recherche sont inacceptables, car ils n'ont qu'un intérêt local.

Articles: Etudes d'une portée générale, constituant des contributions inédites à la science.

Notes: Se distinguent des articles par la portée et le degré d'achèvement plutôt que par la qualité des recherches. Elles peuvent signaler une connaissance nouvelle et significative découlant de problèmes étroitement limités et facilement définis, ou d'importantes découvertes qui méritent d'être publiées avant que des études plus vastes ne soient terminées. Les notes ne doivent pas dépasser 3 pages imprimées (9 pages de manuscrit).

Critiques: Appréciations critiques détaillées de vastes champs de recherches, particulièrement dans les domaines ayant connu de rapides progrès au cours des dernières années.

Perspectives: Essais portant sur une opinion ou une hypothèse relative aux sciences aquatiques qui intéressent professionnels et profanes. Il est préférable de donner des références et d'écrire, si possible, dans un style simple.

Lettres et commentaires: Opinions sur des sujets récemment exposés dans le *Journal*. Longueur maximale préférable: 500 mots.

Disposition générales

Le *CBE Style Manual* (3e éd. AIBS, Washington, D.C. 20016) sert de guide quant à la forme de la présentation. Le *Webster's Third New International Dictionary* et le *Grand Larousse Encyclopédique* font autorité pour l'orthographe des mots. Les titres de périodiques sont abrégés suivant les *Biological Abstracts*. Les références bibliographiques dans le texte doivent suivre le système nom de l'auteur-année. Pour les noms de poissons et leur orthographe, le *Journal* suit les recommandations contenus dans *A List of Common and Scientific Names of Fishes from the United States and Canada* (3e éd. 1970) Publ. spéc. n° 6, American Fisheries Society. Toutes les mesures doivent être conformes au système métrique, de préférence en unités SI.

On devra éviter les *articles en séries*. Un *résumé* doit accompagner chaque texte et doit exposer brièvement, en moins de 250 mots, ce qui a été fait, ce qui a été découvert et les conclusions auxquelles on est arrivé. Le résumé doit être suivi d'un nombre de *mots clés* ne dépassant pas 10.

Chaque texte est soumis d'ordinaire à deux *arbitres*. Nous encourageons les collaborateurs à suggérer les noms d'arbitres possibles.

Les *illustrations* doivent être soumises en triple sous forme de reproductions photographiques. Elles ne doivent pas être montées, brochées ni coupées. Les symboles, lettres ou chiffres doivent se détacher avec précision lorsque l'illustration est réduite à la largeur de la colonne. Les desseins doivent, autant que possible, être groupés pour faciliter l'étude comparative.

L'auteur doit revoir les *épreuves* qu'on lui remet et en corriger les erreurs typographiques. *Il doit éviter de changer le contenu du texte.*

Cinquante *tirés à part* sont fournis gratuitement. Des tirés à part supplémentaires peuvent être achetés au moment de la publication. (Un bon de commande à cet effet accompagne les épreuves.) Les frais sont de $40.00 par page pour la publication d'articles. Quand un auteur soumet un manuscrit, il doit préciser que son institution accepte les frais.

Liste de contrôle pour manuscrits

Une attention particulière aux questions suivantes facilitera l'évaluation des manuscrits par les arbitres et les éditeurs.

Les découvertes, interprétations et conclusions sont-elles bien documentées et pertinentes au but de l'étude?

Les tableaux et figures sont-ils tous nécessaires et agencés pour faciliter les comparaisons? Y a-t-il contradiction entre les tableaux ou les figures et le texte, ou dans le texte même? Est-ce que certaines données devraient être présentées séparément, dans un rapport manuscrit ou dans un dépôt de données? Si vous faites une analyse statistique, la statistique cède-t-elle le pas à la recherche?

Le texte y gagnerait-il en clarté s'il était plus condensé, en tout ou en partie? Contient-il des exposés sommaires au début de chaque section ou paragraphe, et les détails de ces sections et paragraphes sont-ils pertinents au sujet traité? La marche du manuscrit est-elle conforme à la logique énoncée dans l'introduction?

Le *titre* est-il représentatif du contenu du rapport? Le *résumé* donne-t-il l'essentiel des connaissances nouvelles? L'*introduction* se limite-t-elle à une esquisse de la portée, du but et des raisons de l'étude? La revue de la littérature se limite-t-elle à la définition du problème? La section *matériel et méthode* contient-elle le minimum de détails qui permettront aux autres scientifiques de saisir les grandes lignes de l'étude et de juger de la valeur des données? Les généralisations sur les *résultats* sont-elles adéquates? Permettent-elles de distinguer entre constatations et déductions? La *discussion* est-elle limitée à l'interprétation et à l'évaluation des découvertes?

Journal of the Fisheries Research Board of Canada

Guide for Typists Preparing Copy

Read "Guide for Authors" in the current volume of the *Journal*. When in doubt about how to type or arrange details, follow *Journal* practice.

Type the manuscript on white bond paper, not flimsy, preferably size 21.5 × 28 cm (8.5 × 11 inches). If possible, use paper with the lines numbered in the margin. Leave all margins at least 2.5 cm wide.

Double-space all parts of the manuscript, including tables, captions for illustrations, footnotes, and the reference list. Underline only material that is to be printed in italics. Indent the first line of each paragraph in the text and of each footnote.

In titles and headings, capitalize the first letter of each word. For small capitals, use small letters.

Indicate all text footnotes by superscript arabic numerals. Number them consecutively throughout the manuscript, starting with those on the title page. Place each footnote immediately below the reference to it, separating it from the text by horizontal lines.

On the first page type only the title, the authors' names and affiliations, related footnotes and the running head (see Appendix A). Type the abstract with its bibliographic citation on a separate page (see Appendix B). Number all pages beginning with the title page and including those for tables and captions for illustrations.

Have the author check the finished manuscript, and send the ribbon copy and two other clear copies to the Scientific Information and Publications Branch.

Tables: Examine the current volume of the *Journal* to see how tables are set up (see Appendix C).
Note especially the following concerning *numbered tables*:

Type each on a separate page, using paper without the lines numbered.

Number them with arabic numerals.

Use horizontal lines above and below the headings and below the columns, and seldom elsewhere. Never use vertical lines; leave extra spaces instead.

Indicate table footnotes by superscript small letters and type them below the table.

Place the tables after the list of references.

Where each table is first referred to, type in the margin "Table — near here."

Note that *text tables* are not numbered, are typed with the text, and seldom need horizontal lines (see Appendix C).

Illustrations: Send three photographic reproductions (no larger than 21.5 × 28 cm) of each illustration with the manuscript. Type the author's name and figure number on a strip of paper and fasten it to the back of the print. Do not write or type on the illustration itself. Also, type the captions in succession on a separate page.

Where each figure is first referred to, type in the margin "Fig. — near here."

Mathematical Expressions, Equations, and Formulas: Leave three spaces above and below lines that have much material in superscripts, and above and below equations and formulas. In the various expressions, place each component in its correct position (see Appendix D).

Show all symbols clearly. In the margin, spell out Greek letters the first time they occur, and distinguish between the small letter *l* and the numeral *one*, and between the capital *O* and the numeral *zero*. Make other explanatory notes similarly.

References: Check the text to see that all references in the list are referred to. Type the reference list, double-spaced, in the style used in the *Journal* (see Appendix E). Type names of journals in full.

Spellings: About 100 words common in fisheries journals are listed in Appendix F.

Appendices: Appendices A to F are available free from the *Journal*.

Journal de l'office des recherches sur les pêcheries du Canada

Guide pour les secrétaires d'auteurs

Lisez le « Guide des auteurs » reproduit dans l'édition courante du *Journal*. Si vous avez des doutes sur la façon de dactylographier le texte ou d'en agencer les détails, suivez la pratique du *Journal*.

Dactylographiez le manuscrit sur papier blanc de bonne qualité, et non sur papier pelure, mesurant de préférence 21.5 × 28 cm (8.5 × 11 pouces). Si possible, employez un papier avec lignes numérotées en marge. Laissez toujours une marge d'au moins 2.5 cm.

Tout le manuscrit, y compris tableaux, légendes de figures, renvois et références bibliographiques, devra être dactylographié à double interligne. Ne soulignez que les mots qui devront être imprimés en italique. Mettez en retrait la première ligne de chaque paragraphe du texte et de chaque renvoi.

Dans les titres et les en-têtes, n'employez la majuscule que pour la première lettre de chaque mot. Pour les petites majuscules, employez les minuscules du dactylographe.

Indiquez tous les renvois du texte par des chiffres arabes suscrits. Numérotez les renvois consécutivement par tout le texte, à commencer par ceux de la page titre. Placez chaque renvoi directement sous sa référence, le séparant du texte par des lignes horizontales.

La première page ne doit contenir que le titre de la publication, le nom et l'affiliation des auteurs, les renvois appropriés et le titre courant (voir annexe A). Dactylographiez le résumé sur une page séparée (voir annexe B). Numérotez chaque page, à commencer par la page titre, y compris celles qui contiennent les tableaux et la légende des figures.

Voyez à ce que l'auteur vérifie le manuscrit final, et soumettez la première copie ainsi que deux copies lisibles à la Direction de l'information et des publications scientifiques.

Tableaux: Consultez une copie du *Journal* pour la composition des tableaux (voir annexe C).

Portez une attention particulière à ce qui suit au sujet des *tableaux numérotés*:

Dactylographiez chaque tableau sur feuille séparée, vous servant de papier sans lignes numérotées.

Numérotez les tableaux en chiffres arabes.

Tracez des lignes horizontales au-dessus et au-dessous des entêtes et au-dessous des colonnes, rarement ailleurs. Ne tracez jamais de lignes verticales; laissez plutôt des espaces supplémentaires.

Indiquez les renvois dans les tableaux par les minuscules suscrites et placez les renvois au bas du tableau.

Placez les tableaux à la suite des références bibliographiques.

A l'endroit où le tableau est mentionné pour la première fois, indiquez dans la marge « Tableau — près d'ici ».

Remarquez que les *tableaux dans le texte* ne sont pas numérotés, sont dactylographiés avec le texte et ne requièrent que rarement des lignes horizontales (voir annexe C).

Illustrations: Envoyez avec le manuscrit trois reproductions photographiques (21.5 × 28 cm ou moins) pour chaque illustration. Dactylographiez le nom de l'auteur et le numéro de la figure sur une lisière de papier que vous collerez au dos. N'écrivez pas à la main ou à la machine sur la photographie même. De plus, dactylographiez les légendes, dans l'ordre, sur feuille séparée.

A l'endroit où chaque figure est mentionnée pour la première fois, inscrivez en marge « Fig. — près d'ici ».

Equations, formules et expressions mathématiques: Laissez trois espaces au-dessus et au-dessous des lignes renfermant des chiffres ou des lettres suscrits et souscrits, ou encore au-dessus et au-dessous d'équations et de formules. Placez chaque composante des différentes expressions dans la position qui lui est propre (voir annexe D).

Indiquez clairement tous les symboles. Epelez en marge les lettres grecques, la première fois qu'elles apparaissent. Faites une distinction entre la lettre *l* et le chiffre *un*, et entre la majuscule *O* et la chiffre *zéro*. Insérez de la même façon toute autre note explicative.

Références: Vérifiez le texte afin de vous assurer que toutes les références apparaissant dans la liste bibliographique sont bien mentionnées dans le texte. Dactylographiez à double interligne dans le style en usage dans le *Journal* (voir annexe E). Ecrivez les noms des périodiques au long.

Orthographe: Une liste d'environ 100 mots communément employés dans les périodiques sur les pêches est donnée en annexe F.

Annexes: On peut se procurer gratuitement une copie des annexes A à F en s'adressant au *Journal*.

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